MICROBIOLOGICAL REVIEWS, Mar. 1993, p. 1–33
0146-0749/93/010001-33$02.00/0

Vol. 57, No. 1

Bacillus subtilis Sporulation: Regulation of Gene Expression and Control of Morphogenesis†

JEFFERY ERRINGTON

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, United Kingdom

† This review is dedicated to the memory of Peter Butler.

INTRODUCTION

Sporulation as a Developmental System

Endospore formation is the ultimate example of adaptation of bacteria to starvation. The response involves the cooperation of two sister cells in a series of developmental changes culminating in the packaging of one cell into a tough resistant coat. The other cell contributes most of its resources to the process and then lyses. Unlike most adaptive responses in bacteria, sporulation takes many hours and includes major changes in cellular morphology as well as in biochemistry and physiology. Because it also involves the differentiation of two cells that start out with identical genomes, it incorporates two of the main characteristics of developmental processes in all organisms, i.e., temporal change and cellular differentiation. Sporulation has therefore attracted interest not only from bacteriologists and those interested in the practical aspects of spore formation but also from developmental biologists (see reference 158 for a historical perspective). Its advantages in this respect are its relatively simple cellular organization, its experimental tractability, and its excellent genetics.

Although sporulation has been studied as a simple example of development for many years, in the last 10 years or so spectacular progress has been made in isolating the developmental genes and in unraveling their functions and interactions. Indeed, great progress has been made in elucidating the complex genetic program regulating sporulation, and many of the genetic interactions can be explained by the action of transcription factors that have been characterized in vitro. This places sporulation among the best understood of developmental systems and has opened the way to answering some of the most basic questions of developmental biology at the molecular level. Prominent among these questions are the mechanisms responsible for the establishment of cell-specific gene expression and the temporal control of gene expression. Recent work has also demonstrated intercompartmental interactions coordinating development in the two cells and the possible existence of feedback regulation from morphological structure to gene expression.

In this review I update our knowledge of the genes and gene products controlling sporulation and summarize the pattern of interactions between them. The process can be divided into five major phases, four of which are coordinated by sporulation-specific sigma factors, which act as master regulator genes, effectively determining the cell type at any given stage. In the second half of the review I describe the major morphological and biochemical changes that occur during sporulation and discuss what is known about the functions of the genes that bring about these changes.

Previous Reviews

Numerous lengthy reviews have covered the physiological and biochemical aspects of sporulation in detail (see, for example, references 92, 93, 104, 252, 253, and 315). The classical review of sporulation genetics by Piggot and Coote (221) still has much to commend it, especially if read in conjunction with more recent updates (see, e.g., references 179 and 223). Specific aspects of the material treated here are

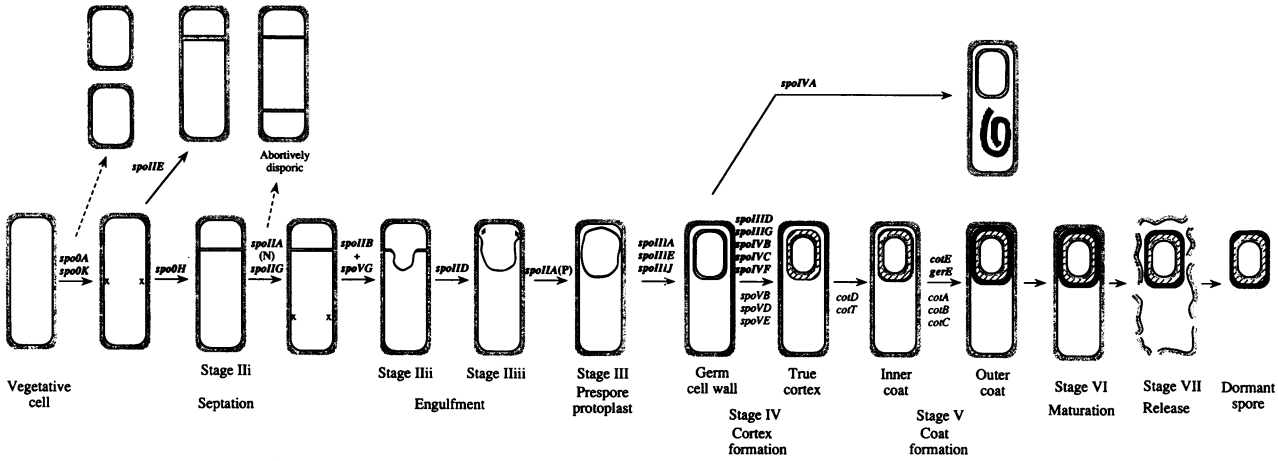

FIG. 1. Morphological events of *B. subtilis* sporulation, and the effects of mutations in various sporulation genes on morphology. The main sequence of morphological events begins with a vegetative cell and ends with the release of a mature spore. The intermediate steps that have been recognized are joined by horizontal arrows. Genes shown in boldface type above the arrows are required for the step indicated by the arrow. Mutations in these genes give a mutant phenotype similar to the cell shown immediately before the arrow. Genes encoding proteins that are known to act at a specific point in the sequence but that do not block development are shown below the arrows. Thus, for example, the *cotT* gene encodes a protein that assembles into the inner spore coat. In a *cotT* mutant the spores are normal in gross morphology and are apparently deficient only in the CotT protein. The locations of potential cell division events that can occur in the presence of certain mutations are indicated by crosses. In such cases, cells with additional septa are formed (indicated by the dotted arrows). Other mutations cause the organism to continue on an abnormal pathway, leading to the formation of the aberrant cells, also shown above the main sequence.

covered in several recent short reviews (66, 72, 105, 163, 178, 200, 276).

Morphological Changes during Sporulation

We are fortunate that in *Bacillus subtilis* the morphological, physiological, and biochemical changes that accompany sporulation have been studied and described in great detail over the last 30 or so years. In the classical electron-microscopic studies of Schaeffer and colleagues (see, e.g., reference 236) seven well-characterized morphological transitions were defined, but, of course, in reality these merge into one another, providing little indication of the underlying mechanisms. Thus, some of the steps originally defined were somewhat arbitrary. Since these pioneering observations of wild-type sporulating cells were made, many developmental mutants have been identified and characterized. Some of these mutants are blocked at discrete points in the normal morphological continuum, and study of these has provided information about intermediate steps in the morphogenic process for which the action of a specific protein is required. Other mutants are directed into pathways producing aberrant structures. Figure 1 shows a schematic summary of the morphological events of sporulation and some of the consequences of mutations in specific genes; the roles of these genes in morphogenesis will be discussed in detail in the main body of the review (see also reference 221).

Although the classical morphological stages of sporulation have been described in detail many times, it is useful at this point to summarize them briefly. Sporulation is generally induced by starvation. In a good growth medium the vegetative rod-shaped cells proliferate in much the same way as most other rod-shaped cells. They double in length and then divide centrally to produce two identical daughter cells. Such vegetative cells were defined by Ryter (236) as being in stage 0 with respect to the sporulation cycle. Sporulation begins with an asymmetric division, producing sister cells that differ markedly in size (Fig. 1). Completion of the specialized spore septum was defined as stage II. (Stage I as originally defined by Ryter [236] is generally no longer recognized because it does not seem to be specific to sporulation and because no mutants arrested at this stage have been found [221].) The smaller prespore is then engulfed by the mother cell in a process now recognized to involve several distinct intermediate steps (see below). When engulfment has just been completed (stage III), the membranes surrounding the cytoplasm of the prespore (now called the forespore) have a very amorphous appearance, presumably because there is no layer of peptidoglycan (PG) to provide them with a defined shape. The distinct ovoid shape of the mature spore begins to become apparent as a layer known as the cortex, a modified form of cell wall (305), is synthesized between the prespore membranes (stage IV). At about the same time that the cortex is being synthesized between the spore membranes, the proteinaceous spore coat begins to be deposited on the outside surface of the spore (stage V). The final period of spore development, termed maturation (stage VI), occurs with little overt change in morphology, but during this period the characteristic properties of resistance, dormancy, and germinability appear in sequence (55, 142). Release of the mature spore by lysis of the mother cell is defined as stage VII.

Sporulation Genes

We shall see during the course of this review that the prespore and mother cell undergo very different physiological and morphological changes during development. This simple system therefore poses several interesting questions of developmental regulation. As a result of the application of the powerful classical and molecular genetic methods available for *B. subtilis* (reviewed in references 45, 65, and 316), most of the regulatory genes determining the patterns of gene expression during sporulation have now been characterized.

In general, the genes involved in sporulation are scattered

around the *B. subtilis* chromosome and are interspersed with genes having no known role in sporulation. I have not provided a systematic list of all known sporulation genes, but Table 1 contains some background information on, and numerous references to, most of the well-characterized genes. The reader is referred to the most recent review of the *B. subtilis* chromosome map for an up-to-date list of genes (220).

***spo* genes.** Classical genetic studies have implicated about 50 genetic loci, designated *spo*, in the control of sporulation. Originally the designation was used for mutations that affected spore formation without any obvious effects on vegetative growth under a variety of conditions (221). Such *spo* loci were named according to the stage at which a mutation in the locus blocked sporulation. Thus, the designations *spo0*, *spoII*, *spoIII*, etc., indicated that mutations in these loci would allow progress to stage 0, stage II, and stage III, respectively (Fig. 1), but not beyond. Loci containing mutations conferring similar phenotypes but mapping at distinct chromosomal positions were distinguished by additional capital letters: *spoIIA*, *spoIIB*, *spoIIC*, etc.

Unfortunately, the definition of *spo* loci is rather loose, and certain errors of mapping and phenotypic characterization have resulted in confusion. The main problem has been that mutations originally assigned to different loci have been found to lie in the same locus. For example, the single mutations defining the *spo0D* and *spo0G* loci are now known to lie in the *spo0B* and *spo0A* loci, respectively (73); the mutations defining the *spoIVD* and *spoIVE* loci were found to be extensive deletions that overlap the *spoIVC* locus (165); and the mutations defining the *spoVH* and *spoVJ* loci (119) are now known to lie in *spoVA* and *spoVK*, respectively (67, 81). Several *spo* loci originally defined by transposon-induced mutations (240) have subsequently been found to lie in preexisting loci, mainly because electron microscopy was not included in the original phenotypic characterization of the transposon mutants (e.g., *spoVL* [44] and *spoVP* [232]). Molecular genetic analysis has revealed that many of the *spo* loci identified in the classical genetic studies are polycistronic (e.g., *spoIIA* [222] and *spoVA* [86]). Thus, the total number of *spo* genes is probably two- to threefold greater than the total number of *spo* loci.

spo genes make up only a proportion of the genes normally expressed during sporulation. Several other categories of genes that participate in sporulation have been identified.

***ger* genes.** Spore resistance and dormancy properties are reversed rapidly by chemicals that act as germinants. Functions involved in the germination response have been identified by *ger* mutations, which interfere with germination and were originally thought to have no effect on spore formation (for a recent review, see reference 198). However, because germination occurs in the absence of de novo gene expression, the functions required for germination must be built into the spore as it develops. It follows that the genes encoding germination functions must be expressed during sporulation. Thus, although several genes specifically involved in germination have been discovered, the distinction between *spo* and *ger* designations is often arbitrary. For example, the *gerE* gene encodes a small DNA-binding protein (39, 123) that regulates gene expression during the later stages of spore coat synthesis (322). Consequently, mutations in *gerE* lead to defects in spore formation (143), but they were first identified on the basis of their effects on germination (197). *gerM* and *gerJ* mutations also seem to have pleiotropic effects (238, 302). Conversely, some *spo* mutants produce spores with altered germination properties (e.g., *spoVIA* [140] and *spoVIB* [141]).

Other sporulation genes. A number of other approaches have been used to identify genes involved in sporulation. Several have been identified on the basis of the products they encode. The abundant small acid-soluble proteins (SASPs) of the spore core and the proteins of the spore coat are encoded by families of genes of which many of the members have been isolated and characterized (see, for example, references 56 and 254). Genes encoding sporulation-associated enzymes have also been cloned, for example, glucose dehydrogenase (170) and alkaline phosphatase (16). The *dci* loci were cloned on the basis of their rapid induction at the onset of sporulation (193).

A further class of genes needed for sporulation is also expressed during growth. These genes include cell division genes such as *ftsZ* and *ftsA* (11, 102, 103, 113) (the latter previously targeted by a "*spo*" mutation: *spoIIN279* [314]) and genes encoding many enzymes (e.g., the *ans* operon, encoding L-asparaginase and L-aspartase [282]). In general, this class of genes has not yet been studied in much detail.

REGULATION OF GENE EXPRESSION DURING SPORULATION

Temporal Control of *spo* Gene Expression

During the last decade or so, many sporulation genes, including almost all of the *spo* genes, have been cloned and characterized. Studies of their regulation, mainly by use of fusions to a *lacZ* reporter gene, have provided information about the temporal sequence of gene activation, with the appearance of β-galactosidase activity indicating the onset of gene expression. These timings can then be related to the various morphological changes described above, determined under similar conditions (209). When measurements of mRNA synthesis have been made, they have generally been in agreement with the results of translational *lacZ* fusion assays (see, e.g., references 76, 186, 246, and 323). Thus, there is as yet little evidence of translational regulation.

Unfortunately, it is sometimes difficult to compare the times of expression of genes measured in different laboratories, because several different approaches have been used to induce sporulation. The nutrient exhaustion method involves growing cells in a rich medium until some (often unknown) component of the medium becomes limiting. This point, the end of exponential growth, is arbitrarily defined as the initiation point of sporulation (t_0). Clearly, in terms of the timing of events during sporulation, the reproducibility of this procedure depends on the accuracy with which the end of exponential growth can be detected, and this is partially dependent on the frequency and accuracy of the culture density measurements. The resuspension method involves harvesting cells from a relatively rich medium and resuspending them in a starvation medium (266). Although this procedure requires two different media, it has the important advantage that the time of resuspension (t_0) provides a fixed point for the initiation of sporulation from which subsequent events can be accurately timed (209). This method is extremely reproducible as long as precise experimental conditions are rigorously adhered to (209). The amount of carryover of growth medium into the starvation medium, the time taken for the harvesting and resuspension steps, etc., can affect the reproducibility of the method. It should also be noted that the time taken for the cell to detect the change in medium composition and respond by initiating sporulation is

TABLE 1. Timing, localization, and transcriptional regulation of sporulation gene expression

Gene or protein[a]	Other name(s)	Map position (degrees)	Onset of expression (min)[b]	Localization[c]	Evidence for localization[d]	Sigma factor	Evidence for sigma factor assignment[e]	Other regulatory effectors[f]	Reference(s)
Preseptation									
abrB	*abs, cpsX, tol*	326	V			σ^A	2	0A(−), AbrB(−)	217, 278
hpr	*catA, scoC*	75	V					0A(−)	215
kinA	*spoIIJ, scoD*	118	V[g]			σ^H	2, 3		7, 212, 216, 226
sin	*flaD*	221	V			σ^A	2		97
spo0B	*spo0D*	241	V[g]			σ^A	2		19, 84
spo0H	*sigH*	11	V			σ^A	2, 3	AbrB(−)	307, 327
spo0K	*opp*	104	V			σ^A	1		213, 235
spoIIIE	*spoIIIB*	142	V			σ^A	2		88
spoIIIJ	*spo0J*	352	V			σ^A	2		68
spo0A	*spo0C, spo0G, spoIIL*	217	V[g]			P_v, σ^A	2		31, 85, 226, 311, 312
			0			P_s, σ^H	2, 3		
spo0E		120	V, 0[g]			σ^A	2		214, 216
spo0F		324	V			σ^A	2		172, 226, 292, 312
			0[g]			σ^{Hh}	2, 3		
dciA			0[i]			σ^A	2, 3	AbrB(−)	259
spo0J		352	0–30						20
spoIIB		244	0–30			σ^H	1		184
spoVG	*0.4 kb*	6	0–30			σ^H	2, 3, 4, 5	AbrB(−)	28, 115, 201, 324, 325
spoIIA	*sigF*	211	40			σ^H	2, 3	0A(+), Sin(−)	76, 182, 218, 308, 309
spoIIE	*spoIIH*	10	30–60			σ^A	2	0A(+), Sin(−)	109, 182, 313
spoIIG	*sigE*	135	0–60[j]			σ^A	2, 5	0A(+), Sin(−)	154–156, 182, 245
Postseptation									
σ^E			60–120[j]	M, P	F				27, 294, 295
spoIID	*spoIIC*	316	80	M	E, G	σ^E	2, 3, 4		57, 132, 233, 268, 273
spoIIIA		220	80	M	G	σ^E	2, 4	IIID(−)	53, 130, 132
spoIVA	*spoVP*	204	80–90	M	G	σ^E	2, 4		53, 232, 267
spoIVF	*bofB, spoVL*	242	60–90			σ^E	2, 4		40, 44
spoVB	*spoIIIF*	239	60–90	M	G	σ^E	1, 4		53, 224
spoVD		133	80	M	G	σ^E	2, 4	IIID(−)	48
bofA			60–90			σ^E	2, 4		134, 230
gerJ		206	60–90						302
gerM		251	90			σ^E	1		238, 261
phoAIII (APase)		50	90	M	I	σ^{Ek}	1		4, 30, 209
nucB (DNase)		227	100	M	I	σ^E	1		4, 74, 300
spoIIID		302	80, 100[l]	M	I, F, G	σ^E	2, 4, 5	IIID(+)	43, 53, 130, 164, 268, 286
spoVK	*spoVJ*	168	110	M	F, G	P_1, σ^E	2, 4	IIID(+)	80, 81, 89, 90
						P_2, σ^K	2		
cotE		150	60–120	M	I	P_1, σ^E	2	IIID(+)	321, 323
						P_2, σ^K	2		
gpr			80, 120[m]	P	E, F[n], R	σ^{Fo}	2, 3, 4		173, 183, 209, 210, 281, 284
Postengulfment									
spoIIIG	*sigG*	135	120	P	I, F, G	σ^{Fo}	2, 3, 4		88, 101, 132, 151, 209, 211, 247, 280
spoVA	*spoVH*	211	150	P	F, G	σ^G	2, 3, 4		53, 77, 132, 199, 207, 283
gdh (GDH)		34	150–180	P	F, R	σ^G	2, 3, 4		82, 94, 153, 204, 228, 283, 284
gerA		289	150–180[p]	P	R	σ^G	2, 3, 4		82
gerB		314				σ^G	2, 4		38
gerD		16	150[p]	P	R	σ^G	2, 3, 4		153
sspA		266	150–180	P	E, R	σ^G	2, 3, 4		91, 128, 187, 207, 283
			120[g,p]						
sspB		65	120[g,p]	P	E, R	σ^G	2, 3, 4		91, 187, 207, 283
sspD		121	120[g,p]	P	R	σ^G	2, 3, 4		187, 207, 283
sspE		65	120[g,p]	P	E, R	σ^G	2, 3, 4		91, 187, 207, 283
spoIIIC	*sigK, spoIVD*	227	150	M	F				165, 297
spoIVCB	*spoIVE*	227	180			σ^{Kq}	2, 3	IIID(+), GerE(−)	162, 165, 166
spoIVB		213	240	P	E, R	σ^G	2, 4		43, 299
spoVE		133	240	M	G	σ^K	1		53, 117, 118
spoVF (DPA)	*dpa*	148	240	M	F	σ^K	2	GerE(−)	5, 50, 301
sspF	*0.3 kb*	6	240	P	F	σ^G	2, 4		175, 208
gerE		253	240	M	E, I	σ^K	2		41

Continued on following page

TABLE 1—*Continued*

Gene or protein[a]	Other name(s)	Map position (degrees)	Onset of expression (min)[b]	Localization[c]	Evidence for localization[d]	Sigma factor	Evidence for sigma factor assignment[e]	Other regulatory effectors[f]	Reference(s)
cotA	*pig*	52	240	M	I	σ^K	2	GerE(−)	239, 322
cotD		200	240	M	I	σ^K	2, 3	GerE(+), SpoIIID(−)	162, 323
cotF		349	240[r]	M	I	σ^K	2		4, 17
cotT		114		M	I	σ^K	2	GerE(−)	8
cotB		290	360	M	I	σ^K	2, 3	GerE(+)	322, 323
cotC		168	360	M	I	σ^K	2, 3	GerE(+)	322, 323

[a] Genes are listed in approximate order of expression. They are tentatively divided into three sections according to whether their time of expression precedes septation (preseptation), begins after septation but before engulfment (postseptation), or begins only after engulfment is complete (postengulfment) (209).

[b] When possible, the times quoted (minutes after initiation of sporulation) are from experiments in which the resuspension method was used (266), since these give the most accurate and reproducible timings. Possible anomalies are noted.

[c] M, mother cell; P, prespore.

[d] E, immunoelectron microscopy with anti-β-galactosidase against protein expressed from a *lacZ* fusion, except for σ^E, for which the antibodies were raised against the protein itself, and *sspA*, *sspB*, and *sspE*, for which both types of antibody were used; F, fractionation on the basis of differential susceptibility to mechanical shearing forces, with β-galactosidase activity used except where otherwise indicated; G, genetic method of De Lencastre and Piggot (53) or Illing et al. (132); I, inference from function of protein; R, detection based on occlusion of β-galactosidase activity in the developing prespore.

[e] 1, sequence searching only; 2, sequence similarity based on a transcription start site determined by primer extension or nuclease protection; 3, transcription from the correct start site in vitro; 4, induction of expression by synthesis of σ in vegetative cells; 5, allele-specific suppression of a promoter mutation by a mutation altering the DNA recognition region of the cognate sigma factor.

[f] Where there is reasonable evidence for a direct interaction, e.g., binding of the protein to the DNA in vitro, or tight correlation between time of activation of regulator and effect on gene expression. +, positive regulation; −, negative regulation.

[g] Sporulation by nutrient exhaustion.

[h] Transcribed by $E\sigma^H$ (226) rather than $E\sigma^B$ as stated in the original paper (172).

[i] Sporulation induced by decoyinine.

[j] Timing of pro-σ^E and mature σ^E, measured by Western immunoblot from exhaustion culture.

[k] The *phoAIII* gene appears to have a σ^E promoter, but at least one other gene contributes to sporulation alkaline phosphatase activity (16).

[l] An initial phase of low-level expression occurs after 80 min; the main phase begins at about 100 min (268). Very different timing was observed by Kunkel et al. (164), who noted onset of expression after 150 min.

[m] An initial phase of low-level expression occurs after 80 min; the main phase begins at about 120 min (209).

[n] Fractionation on the basis of immunological detection; experiment done on *B. megaterium* (173).

[o] These promoters can also be utilized by $E\sigma^G$ in vitro and in vivo (280, 284).

[p] Coincided with the onset of glucose dehydrogenase activity.

[q] This promoter can probably also be utilized by $E\sigma^E$ (166).

[r] From comparison with *cotD* transcription in a primer extension experiment (42).

unknown. A third method, based on induction of sporulation in rich medium by the antibiotic decoyinine, has been used occasionally. This again provides a defined t_0, but the efficiency of sporulation is relatively poor (195).

Table 1 lists the time of onset of expression for various genes; when possible, data obtained by the resuspension method are reported. The genes are tentatively divided into three groups according to whether they begin to be expressed preseptation (about 65 min after resuspension [209]), postseptation (between 65 and 105 min [209]), or postengulfment (after about 105 min [209]). Some genes, particularly but not exclusively the *spo0* genes, are expressed in vegetative cells. A subset of these are turned off at initiation (e.g., *spo0B*). Others show increased expression within the first 30 min. Studies of this phenomenon suggest that the genes have dual promoters, one promoter driving expression during vegetative growth and the second taking over or boosting expression at or around initiation (e.g., *spo0A*). Vegetative expression of *spo0* genes is not surprising, since their products are envisaged to control the initiation of sporulation. However, two genes in which mutations have an apparent effect on sporulation only at stage III, *spoIIIE* and *spoIIIJ*, are also expressed vegetatively. The reason why these genes are turned on so far in advance of any obvious requirement for their products is not clear.

Differences between the timings measured in different laboratories preclude a detailed temporal classification of the various genes shown in Table 1. However, it is clear that the transcriptional regulation of sporulation is complex, involving a series of sequential changes occurring over a period of at least 6 h after the onset of sporulation.

Cellular Localization of Gene Expression

As discussed above, the core of the mature spore contains a number of highly abundant proteins, and these, for topological reasons, must be made within the developing forespore. Conversely, the outside surface of the spore carries at least a dozen coat proteins that, for topological reasons, must be made in the mother cell. Several approaches have been used to determine the cellular localization of the expression of other genes during sporulation. Initially this was done by biochemical fractionation methods (6, 63, 83). Once the cortex begins to form (stage IV; about t_4), the forespore becomes sufficiently robust to withstand mechanical shearing forces that fragment mother cells and nonsporulating cells. The particulate (forespore) and soluble (mother cell) fractions can then be assayed separately for the presence of specific proteins or for the β-galactosidase activity from *lacZ* fusions (see, e.g., references 77 and 258). This procedure works quite well for genes that are expressed during the later stages of sporulation but not for genes expressed before about t_4 (when resistance to mechanical shearing begins to appear in the forespore). Thus, the method can be used to demonstrate that a gene is expressed inside the forespore, but it cannot distinguish between mother cell expression and early forespore expression. A related approach relies on the differential sensitivity of mother cells and forespores to lysozyme or to permeabilizing agents such as toluene (see, e.g., reference 187). This method is simple but again is applicable only when gene expression occurs after the appearance of lysozyme resistance or impermeability. Expression of β-galactosidase in

the forespore can be demonstrated easily by purifying mature spores and then either disrupting them biochemically (187) or permeabilizing them by inducing germination (see, e.g., reference 77); however, the results tell us little about expression in the mother cell.

Recently, immunoelectron microscopy has been used to visualize the locations of specific proteins or of the β-galactosidase produced from *spo-lacZ* fusions (see, e.g., references 57 and 91). This approach has two advantages: it is direct, and it can be applied to genes that are expressed immediately after septation. A disadvantage lies in the limited sensitivity of the method. For weakly expressed genes it has been necessary to amplify the reporter gene construction to achieve detectable levels of antigen (57, 183), and it is possible that amplification perturbs the regulation of the gene, so that the results might not reflect the in vivo situation.

Genetic tests provide a simple but indirect alternative to the biochemical methods. De Lencastre and Piggot (53) used transformation to generate mosaic sporangioles (sporulating organisms with one mutant and one wild-type chromosome). The phenotypes of the progeny of spores selected on the basis of heat resistance depend on whether the gene has to be expressed in the forespore compartment. If forespore expression is necessary, all of the progeny obtained will be genetically Spo^+. However, if the gene is required only in the mother cell, sporangioles with a mutant allele in the forespore and a wild-type allele in the mother cell can form resistant spores, whose progeny are Spo^-. A similar approach based on plasmid excision has been described more recently (132). These methods reveal whether the gene is required in the forespore or the mother cell, but they have several limitations. First, they must be used only for genes that are expressed and required after septation. Second, leaky mutations can give rise to Spo^- progeny even if the gene is required in the forespore. Finally, it should be borne in mind that these methods do not provide information about the actual location of expression; a gene might be expressed in both cells but required in only one. Nevertheless, the method is rapid, and the results, with no published exception, support those obtained by more direct measurements of gene expression.

Another related method has been described by Dancer and Mandelstam (46, 47). They showed that the defective sporulation of certain Spo^- mutants could be rescued by converting them to protoplasts at about stage III and fusing them to protoplasts made from wild-type cells. Again, the results of these experiments indicated that expression of certain genes in the mother cell was sufficient to allow sporulation to occur. However, they suffer from several of the caveats that have been outlined for the methods based on formation of mosaic sporangioles, as described above. Protoplast fusion can also be applied only to genes that are expressed after stage III, because conversion to protoplasts before this time is incompatible with sporulation (47).

Table 1 summarizes the results of the localization experiments that have been reported. Perhaps surprisingly, all of the genes turned on after the separation of prespore and mother cell compartments seem to be expressed in only one of the two cell types. The formation of σ^E may also be mother cell specific, but different experimental methods have given contrasting results. Cell fractionation studies indicated that the proteolytically processed active form of σ^E was present in both the forespore and the mother cell (27), whereas immunoelectron microscopy (57) and a variety of other experiments (reviewed in reference 72) suggest mother cell-specific localization, at least for the genes controlled by σ^E. It is of course possible that σ^E processing occurs in both cells but that the sigma is active only in the mother cell, but this would require an additional level of regulation of sigma factor activity.

It is clear that although the forespore and mother cell compartments start off with identical genomes, they activate different sets of genes during development. Comparison of the data on timing and localization in Table 1 reveals that there are temporal changes in gene expression in both cells, and hence there are even more changes in transcriptional specificity during sporulation than was made apparent by examining the timing of gene expression. The way in which differential programs of gene expression are set in motion after cell division is one of the most interesting and challenging basic problems of developmental biology. This problem has been partly solved for *B. subtilis* by charting the interactions between the sporulation genes, thus identifying not only the earliest cell-specific transcription factors but also the factors that regulate their activities.

Dependence Patterns

The overall pattern of genetic interactions between sporulation genes has been elucidated by measuring the dependence of expression of a series of genes on the products of other genes. The main method used has been to introduce a *spo-lacZ* fusion into each of a series of otherwise isogenic *spo* mutant strains and to measure β-galactosidase synthesis. If the gene fused to *lacZ* is dependent on prior expression of the gene containing the mutation, β-galactosidase production is reduced or abolished. A large number of measurements of this sort have now been made, and I have attempted to summarize them in a greatly simplified form in Table 2. Several important caveats must be attached to the information presented in Table 2. For some loci, many different mutant alleles have been used, not all of which are well characterized. Although most of the results are based on the use of *spo-lacZ* fusions, some were translational fusions and others were transcriptional. Other data were obtained by Northern (RNA) blot analysis. The main anomalies and inconsistencies are listed in the footnotes to Table 2. Despite these caveats, a distinct, internally consistent pattern is beginning to emerge, and 12 different dependence classes have been distinguished in Table 2. The members of each class generally show common timing and localization (compare with Table 1). Thus, the class comprising *spoIIA*, *spoIIG*, and *spoIIE* is expressed in the preseptational cell before septation. Class 3 genes are all expressed after about 80 to 90 min, at the same time as σ^E appears, and in the mother cell compartment. Genes in classes 4 and 5 differ from those in class 3 only in their responses to *spoIIID* mutations: class 4 genes show increased expression in *spoIIID* mutants, whereas class 5 genes show reduced expression. The "early" prespore-specific genes, *gpr* (class 6) and *spoIIIG* (class 7), are placed in separate classes mainly on the grounds of their different responses to *spoIIG* mutations. *spoIVB*, *spoVA*, *gdh*, *gerA*, *gerD*, and the various *ssp* genes are all expressed specifically in the forespore and after about 150 min (class 8). The later mother cell-specific genes fall into four classes, depending mainly on their responses to *gerE* mutations. Class 10 genes are not influenced significantly by *gerE* mutations, class 11 genes are overexpressed in a *gerE* mutant, and class 12 genes are dependent on *gerE*.

The dependence of one gene on another implies that the mutation eliminates the synthesis or activation of a factor

TABLE 2. Dependence patterns for the expression of various sporulation genes

Expression of gene[a]	Reference(s)	Dependence class	Effect of mutation in[b]: *0A 0B 0H 0K*	*0E 0F 0J*	*IIA*(N) *IIE*	*IIG*	*IIA*(P)	*IIB*[d]	*IID IIIA IIIG IIIJ*	*IIID*	*IIIE*	*IVA*	*IVB IVC IVF*	*gerE*	Transcriptional regulation[c]
spoVG	325	1	−	(+)[e]	+	+	(+)	(+)	+	(+)	(+)	(+)	(+)	(+)	σ^H AbrB(−)
spoIIA	76, 246, 308	2	−	− [f]	+	+	+	+	+	+	+	+	+	+	σ^H Spo0A(+)
spoIIE	109														
spoIIG	34, 155, 294														
σ^E	4, 145, 295	3	−	−	−	−	+	+[g]	+	+	+	+	+	+	σ^E
spoIID	32, 233														
spoIVA	232, 267														
spoIVF	44														
APase	4, 36, 74, 219														
DNase	4, 74														
spoIIIA	130	4	−	(−)	−	−	+	±	+	++	+	(+)	(+)	(+)	σ^E SpoIIID(−)
spoVB[h]	224														
spoVD	48														
bofA[h]	134														
spoIIID	164, 268	5	−	−	−	−	+[i]	+	+[j]	±	+	+	+	+	σ^E SpoIIID(+)
spoVK	80, 81														
cotE	323														
gpr	183, 209, 284	6	(−)	(−)	−	±	−	±	±	+	−	(+)	+	(+)	σ^F
spoIIIG	88, 151, 209	7	−[k]	(−)	−	−	−	±[l]	±[m]	+	−[m]	(+)	+	(+)	σ^{Fn}
spoIVB	43	8	−	−	−	−	−	−	−[o]	±[p]	−	+	+	+	σ^G
spoVA	77														
gdh	36, 187, 219														
sspA to *sspE*	187, 188														
sspF	208, 265														
gerA	82														
gerD	153														
spoIVC[q]	166, 297	9	−	−	−	−	−	−	±[r]	−	±	±	±	+	σ^E/σ^K, SpoIIID(+), GerE(−)[s]
σ^K	180	10	−	(−)	−	−	(−)	−	−	−	−	±	−	+	σ^K
gerE	41														
spoVF	50	11	−	−	−	−	(−)	−	−	−	−	±	−	++	σ^K GerE(−)
cotA	239														
cotB	323	12	(−)	−	−	−	−	−	−	−	−	±[t]	−	−	σ^K GerE(+)
cotC	323														
cotD	323														

[a] Expression of various genes, or processing of pro-σ^E or pro-σ^K (rows), usually measured by *lacZ* fusions, has been tested in a range of mutant backgrounds (columns). The levels of expression shown are relative to that of wild-type cells. −, <20% wild type; ±, 20 to 49% wild type; +, 50 to 200% wild type; ++, >200% wild type. Parentheses indicate that none of the group have been tested in that particular mutant background and the result shown is speculative. Various genes and mutants are grouped together on the basis of similar behavior. The groupings should be regarded as tentative in the absence of detailed molecular analysis of regulation or knowledge of the action of the gene product. When a particular difference was observed between different members of a given group, the circumstances are cited in a footnote. Mutations in a number of *spoV* and *spoVI* genes (*spoVA*, *spoVB*, *spoVC*, *spoVD*, *spoVE*, *spoVF*, *spoVK*, *spoVIA*, *spoVIB*, and *spoVIC*) have also been tested extensively but found to have mainly insignificant effects. No attempt has been made to define the dependences of genes expressed in vegetative cells.

[b] For clarity, the *spo* prefixes have been omitted.

[c] Likely transcriptional effectors responsible for regulation of the class of genes (from Table 1). (+), positive regulation; (−), negative regulation.

[d] In most published experiments the strain used to test dependence on *spoIIB* probably also contains a *spoVG* mutation (184).

[e] The *spo0J93* mutation had no effect on *spoVG-lacZ* expression (325).

[f] The *spo0J93* mutation gene did not block expression of *spoIIG* (155), but this experiment involved induction of sporulation by decoyinine.

[g] *spoIVA* expression was only 43% in a *spoIIB* (*spoVG* double) mutant (267).

[h] *spoVB* and *bofA* are included tentatively on the basis of their probable transcription by $E\sigma^E$ and overexpression in the presence of mutations in *spoIIID* (134, 224).

[i] *spoVK* expression was only 17% in a *spoIIAC*(P) mutant (80).

[j] *spoVK* expression was reduced to 48 or 25% by mutations in *spoIIIA* and *spoIIIJ*, respectively (80), probably because of effects on the σ^K-dependent P_2 promoter (89).

[k] *spoIIIG* expression occurs in a *spo0H* mutant, but this is probably an indirect effect of the block in expression of the *spoIIAB* gene (227).

[l] *spoIIIG-lacZ* expression in a *spoIIB* mutant has been reported as 8% (151) and 51% (209).

[m] Results strongly depend on the location of the *spoIIIG-lacZ* fusion (88, 151, 209, 280, 281).

[n] Transcription from the *spoIIIG* promoter is probably mainly due to $E\sigma^F$ (211, 280), but it is blocked by mutations that inactivate σ^E, so an additional level of transcriptional regulation is likely (209).

[o] The reported effects of *spoIIIA* mutations on prespore-specific gene expression vary over a wide range.

[p] Results are relatively variable. Particularly low expression of *spoIVB* (3 to 31%) is found in this background (43).

[q] *spoIIIC-lacZ* (297) behaved somewhat differently from *spoIVCB-lacZ* (166), but the discrepancy is probably due to the site-specific recombination event required for formation of the composite *sigK* gene (165, 275) (see text).

[r] Expression of *spoIVCB-lacZ* was blocked by the *spoIIIJ87* mutation (<5% of wild type), and results with the *spoIID* mutations ranged from 13 to 62%, depending on the mutant allele used (166).

[s] The *spoIVCB* promoter exhibits unusually complex regulation. It can probably be transcribed by RNA polymerase containing either σ^E or σ^K, and it is additionally regulated by both SpoIIID and GerE (162, 166, 322).

[t] Effect is strongly dependent on the medium used to induce sporulation (323).

necessary for expression. A small number of dependences are due to direct regulatory effects, for example by the product of one gene acting directly on the promoter of the other to stimulate transcription. However, most effects are indirect and are due to the heirarchical organization of the program. As will become clear below, many of the genes in which mutations can interrupt the genetic program encode regulatory proteins. However, not all do; some seem to have structural roles in the execution of a morphological event. The apparent dependence of gene expression on a morphological event could indicate the existence of feedback regulation from morphological structure to gene expression as first suggested by Stragier et al. (273). However, there are alternative explanations: the formation of an aberrant structure could have an indirect effect on gene expression by impairing the viability of the cell, for example.

It should be noted that several *spo* genes do not appear in Table 2 because mutations in them have little or no effect on expression of the genes that have been measured (see Table 2, footnote *a*). These mutations seem mainly to affect structures that are formed relatively late in sporulation. Perhaps if feedback mechanisms of the sort discussed above exist, they operate only during the early stages of morphogenesis.

Resolution of the complex pattern of interactions between such a large number of genes represents a considerable achievement, and the extent of our understanding of the interactions between sporulation genes is not matched by any other developmental system. However, this establishment of dependence patterns represents only one facet of our knowledge of the regulation of sporulation gene expression. Several of the genes in Table 2 encode transcription factors that have been isolated and characterized in vitro, and it is now possible to explain, at least tentatively, the molecular basis for all of the distinct dependence classes.

Transcription Factors

The most significant changes in gene regulation during sporulation are brought about by the appearance of new sigma factors, which confer new promoter specificities on RNA polymerase, thereby activating collections of previously inactive genes (176, 177, 200, 276). In addition to the major vegetative sigma factor, σ^A, at least five minor sigma factors contribute to the transcriptional regulation of gene expression during sporulation: σ^H (28, 59), σ^E (111, 274, 294), σ^F (70, 270, 283), σ^G (151, 192, 283), and σ^K (162). For virtually all of the sporulation genes described above, there is at least a tentative indication of the form of RNA polymerase responsible for transcription. The types of evidence linking a specific form of RNA polymerase to a given gene fall into four categories. The first is sequence similarity. Most sigma factors recognize promoters in the form of conserved sequences centered at about −10 and −35 with respect to the transcription start site (all of the sigma factors implicated in the control of sporulation appear to fall into this class [116]). When a number of promoters of a given specificity have been identified, sequence comparisons reveal consensus sequences that can be useful in identifying other promoters of the same class. These can be identified tentatively by simply scanning the DNA sequence of a promoter region or more reliably when the apparent transcription start site has been determined by nuclease protection (14) or primer extension (133) methods. The second type of evidence is transcription in vitro from the correct in vivo start point with purified RNA polymerase. This provides evidence that a particular form of RNA polymerase could be responsible for transcription from a specific promoter (201). The third is the use of an inducible promoter to induce synthesis of a sporulation-specific sigma factor in vegetative cells (273). Immediate induction of expression of a target gene again suggests that the sigma factor is at least capable of directing transcription from the promoter. The fourth is allele-specific suppression of promoter mutations by mutations affecting the promoter recognition regions of the cognate sigma factor (96, 256, 324).

The evidence linking specific forms of RNA polymerase to the promoters of various sporulation genes has been obtained in many laboratories and is summarized in Table 1. Genes expressed vegetatively, or in the first 1 h of sporulation (and therefore before septation), are transcribed by RNA polymerase containing either the major vegetative sigma factor, σ^A (functionally equivalent to the major sigma factor, σ^{70}, of *Escherichia coli*), or a minor sigma factor, σ^H. After septation, four new sporulation-specific sigma factor activities appear. It is apparent from Tables 1 and 2 that each of these sigma factors controls one or more blocks of genes exhibiting similar dependence patterns: the genes transcribed by $E\sigma^E$ all fall into classes 3, 4, and 5 in Table 2; σ^F directs transcription of the *gpr* and *spoIIIG* genes which make up classes 6 and 7; σ^G controls the genes in class 8 (it may also contribute to the transcription of genes in classes 6 and 7 [see below]); and σ^K controls those in classes 9 to 12. Thus, some of the boundaries between dependence classes reflect differences in the form of RNA polymerase responsible for transcription.

When not only the timing but also the localization of expression of the genes transcribed by each sigma factor is tabulated, it becomes apparent that each sigma factor controls the transcription of genes in a specific cell type over a relatively specific period. For the first 1 to 2 h after septation, transcription in the prespore and mother cell is directed by $E\sigma^F$ and $E\sigma^E$, respectively. During the later stages of development, $E\sigma^G$ and $E\sigma^K$ are responsible for transcription in the forespore and mother cell, respectively. From the developmental point of view, the sporulation-specific sigma factors thus act as master regulatory proteins determining cell type, in much the same way as has been postulated for certain proteins controlling differentiation in higher eukaryotes (e.g., the MyoD gene controlling myogenesis [285]). Transcriptional regulation of sporulation can therefore be divided into five discrete stages, both temporal and spatial, each demarcated by the action of a specific sigma factor (Fig. 2) (for reviews, see references 64, 66, 178, 200, and 276).

In addition to the sigma factors, several other proteins are known to exert positive and/or negative control over the transcription of sporulation genes and thus to influence the pattern of dependences summarized in Table 2. The SpoIIID and GerE transcription factors, in particular, further subdivide the genes in the σ^E and σ^K regulons into discrete temporal classes, thus accounting for the distinctions between genes in classes 3, 4, and 5 and classes 10, 11, and 12, respectively (Table 2, final column). As discussed by Zheng and Losick (323), the mother cell line of gene expression, at least, seems to involve a heirarchical cascade of regulatory effectors, in the sequence σ^E, SpoIIID, σ^K, and GerE. Each factor activates a new temporal class of genes and is necessary (although never sufficient) for synthesis of the next transcription factor. Further details of the functions of each transcription factor will be described in the appropriate sections below.

Remarkable progress has clearly been made in working

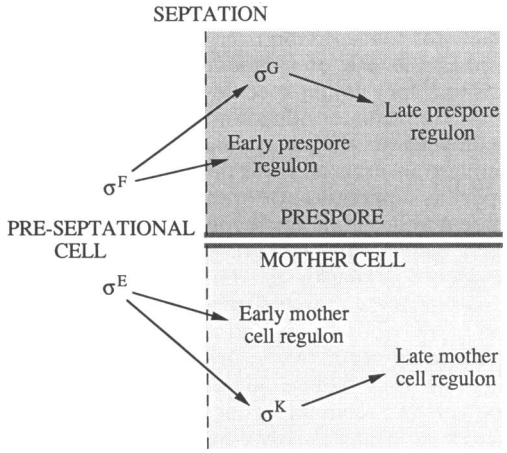

FIG. 2. General functions of the sporulation-specific sigma factors. σ^E and σ^F are encoded by genes expressed in the preseptational cell, i.e., before the formation of the spore septum. After septation each sigma factor appears to control the transcription of genes in a specific cellular compartment, as shown. Among the genes transcribed by σ^E and σ^F are genes encoding further sporulation-specific sigma factors directing the transcription of collections of genes determining the later stages of development in each cell. In reality, the regulation of each sigma factor is under complex control, as shown in subsequent figures. The shading used to distinguish prespore- and mother cell-specific gene expression from that of the preseptational cell is retained in several subsequent figures.

out the transcriptional regulation of sporulation gene expression. However, most sporulation genes do not act directly as transcriptional regulators but instead contribute to the fabrication of the structures of the spore, either enzymatically or structurally. In the remaining part of the review, I discuss what is known about how the key changes in the transcriptional apparatus are regulated and coordinated with the developmental sequence and how the products of the known sporulation genes help to bring about the biochemical and morphogenic changes. The order of the following sections roughly reflects the temporal order of the events in sporulation.

INITIATION OF SPORULATION

Decision To Sporulate

The initiation of sporulation is complex, and at least three types of input are integrated by the cell before it initiates sporulation.

Nutritional signal. The nutritional factors that induce sporulation have been reviewed in detail previously (92, 262). Starvation for sources of carbon, nitrogen, or phosphorus can induce sporulation; good carbon sources, such as glucose, repress it. Freese showed, in a series of incisive experiments, that the intracellular concentration of GTP (or GDP) probably represents the key effector of the nutritional signal (92). However, the way in which information about the intracellular concentration of GTP is acquired by the cell and transmitted to the transcriptional apparatus is not yet understood.

Population density. Grossman and Losick (106) observed that sporulation could not be induced efficiently in cells maintained at a low population density. However, efficient sporulation was obtained when cells were suspended at low density in medium previously conditioned by growth of cells at high density. The effect was not due to depletion of an essential growth factor. Rather, it seemed that vegetative cells grown to a relatively high density produced a substance, possibly an oligopeptide, that is necessary for efficient sporulation. Production of this substance, provisionally called EDF-1 (extracellular differentiation factor 1), depends on several of the *spo0* genes, particularly *spo0A*, *spo0B*, and *spo0H*. The oligopeptide transport system (*opp*) of *B. subtilis* was identified by mutations originally designated *spo0K*, which are simultaneously defective in sporulation and in the uptake of cell wall oligopeptides (213, 235). It thus seems possible that the cell uses the uptake of wall oligopeptides as an indicator of cell wall expansion and hence of growth rate. However, how the cell could sense the rate of oligopeptide uptake, or interpret this information, is unknown.

Cell cycle. The early part of sporulation clearly comprises a modified cell division (120) (see below). Mandelstam and coworkers demonstrated that entry into the sporulation cycle can occur only at a specific point in the cell division cycle. Although the link between the cell cycle and initiation is well established (33, 51, 52, 60, 157, 181), the way in which the cell monitors progress through the cell cycle and transmits this information to the transcriptional apparatus is obscure. There is an obvious overlap with the general problem of vegetative division (not only in prokaryotes), and this area is thus likely to attract a good deal of interest in the near future.

Transcriptional Regulation of the Initiation of Sporulation

Once the appropriate intrinsic and extrinsic conditions for sporulation have been met, the initiation of sporulation occurs. This involves a modified, highly specialized cell division (120) and hence a series of morphological changes different from those that occur in vegetative cells. These events are directed, at least in part, by changes in the transcriptional apparatus of the cell. Two transcriptional regulators, σ^H and Spo0A, play key roles in initiation. Several additional proteins participate, mainly by controlling the activity of Spo0A. The regulation of these various factors and their interactions are described below. Both σ^H and Spo0A exert control over various stationary-phase responses, such as the development of genetic competence and the synthesis of extracellular degradative enzymes, which occur in addition to or instead of sporulation, under certain conditions. A full discussion of these responses is beyond the scope of this review, but several excellent recent reviews covering this subject are available (58, 160, 298).

spo0H. σ^H is encoded by the monocistronic locus *spo0H* (59). The gene has a σ^A-dependent promoter and is expressed weakly in mid-exponential-phase cells. Transcription of *spo0H* gradually increases, reaching a peak at about t_0 (307), but substantial transcription by $E\sigma^H$ does not occur until after the induction of sporulation at t_0 (115). Thus, posttranscriptional control mechanisms, as yet poorly characterized, ensure that the genes controlled by σ^H are not expressed until stationary phase.

A considerable number of genes controlled by $E\sigma^H$ (*csh* genes) have been identified by Jaacks et al. (138). All showed *spo0H*-dependent induction from about t_0, but, surprisingly, mutations in only two of these affected sporulation and then only slightly. Thus, σ^H probably controls genes involved in a range of activities that have not yet been discovered.

Nevertheless, there is evidence that at least one important sporulation-specific operon, *spoIIA*, is controlled by σ^H.

***spo0A* and the phosphorelay system.** The Spo0A protein plays a pivotal role in the initiation of sporulation. The *spo0A* gene is transcribed from two promoters, P_v and P_s. P_v is recognized by $E\sigma^A$ and apparently provides a basal level of Spo0A in vegetative cells. P_s is controlled by $E\sigma^H$ and as such gives an increased burst of Spo0A at the onset of sporulation (31, 311). Spo0A negatively regulates various genes, principally, in this context, *abrB* (217, 277), which is in turn an important negative regulator of various stationary-phase responses, although mainly it regulates genes that are not concerned directly with sporulation (see below). Positive regulation by Spo0A is responsible for the activation of several key sporulation-specific genes, notably those of the *spoIIA* (218), *spoIIG* (244, 245), and *spoIIE* operons (313).

The Spo0A protein belongs to a large family of bacterial proteins called response regulators (reviewed by Stock et al. [269]). These proteins generally have two domains. The N-terminal domain is highly conserved, and an invariant aspartic acid residue is the target for phosphorylation by one or more cognate histidine protein kinases. Phosphorylation controls the activity of the C-terminal domain, which, in Spo0A, is thought to be responsible for DNA-binding and gene regulation activities (218). Although Spo0A, even in the unphosphorylated form, can bind to DNA and repress transcription (244, 277), its binding is enhanced by phosphorylation and its positive regulatory functions are dependent on this (218).

Phosphorylation of Spo0A is thus the key to its control over the initiation of sporulation. Phosphate is transferred to Spo0A by a complex mechanism involving histidine protein kinases (sensor or transmitter proteins) and at least two intermediate phosphate carriers. The interactions between the various participants have recently been reproduced in vitro by Burbulys et al. (26). At least two histidine protein kinases encoded by the *kinA* (*spoIIJ*) and *kinB* genes can transfer phosphate to the Spo0F protein, which can transfer the phosphate to a second intermediate carrier, Spo0B. Spo0B is the immediate donor of phosphate to Spo0A (Fig. 3).

This intricate pathway would allow various inputs from internal and external environmental sensors to control the level of Spo0A-P (26, 105). Clearly, the KinA and KinB kinases could respond to different signals. Increased activity in either of these proteins would tend to increase the flux through the phosphorelay. However, the nature of the inputs to which they respond has not yet been identified. Other inputs could control the flux through the subsequent steps by acting on the intermediate carriers Spo0F and Spo0B. The Spo0E and Spo0K proteins seem to act on the phosphorelay, although their precise targets are not yet clear. The Spo0K proteins have already been discussed in relation to the "extracellular" control of sporulation. The 10-kDa Spo0E protein seems to act as an inhibitor of sporulation, but mutations eliminating the C-terminal portion of this protein block sporulation (214, 216). Presumably, the N-terminal part of Spo0E is an inhibitor acting at some point in the phosphorelay (*spo0E* mutations can be bypassed by certain mutations in *spo0A* [85, 121, 264]) and the C-terminal region modulates the activity of the inhibitor, presumably in response to some signal. Again, the nature of this signal is unknown.

The *spo0B* operon contains a second gene, *obg*, encoding a GTP-binding protein that is essential for vegetative growth (293). Because the *spo0B* and *obg* genes are cotranscribed,

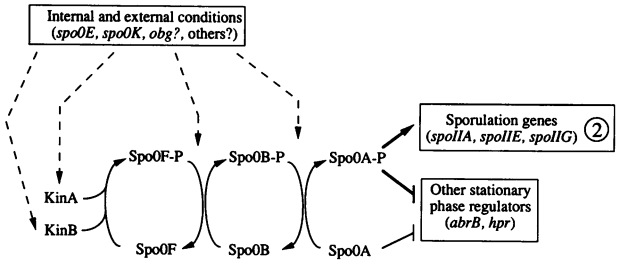

FIG. 3. Control of stationary-phase gene expression by the phosphorelay system. At least two histidine protein kinases, KinA and KinB, can transfer phosphoryl groups to the Spo0F protein in response to as yet unknown signals, perhaps internal or external nutritional conditions (26). The phosphoryl group is then transferred via Spo0B to the Spo0A transcription factor (26). Phosphorylated Spo0A positively regulates the expression of several key early sporulation-specific genes (indicated by the line ending in an arrowhead) (218, 245, 313). The number 2 indicates that these genes are denoted class 2 in Table 2. Spo0A-P also negatively regulates at least two genes controlling various stationary-phase responses (indicated by the line ending in a bar) (215, 277). The nonphosphorylated form of Spo0A is also a weak negative regulator, as suggested by the thin line. Several other gene products are thought to influence the flux of phosphate through the phosphorelay, as indicated by the dashed lines. Their precise targets in the pathway are not yet known, but, in principle, they could act at any one of the four steps shown (26, 105).

Trach and Hoch (293) have suggested that the *obg* gene product may be the crucial protein mediating the nutritional regulation of sporulation, perhaps by sensing the GTP levels in the cell and transmitting this information to the phosphorelay via Spo0B. Alternatively, it might be involved in the cell cycle signal (26).

Elucidation of the phosphorelay system was an important achievement. The existence of various other proteins that seem to act via the phosphorelay shows in principle how information from many different sources can be integrated to determine the choice between proliferation and differentiation. Again, our knowledge of this system may have important consequences for thinking about the ways in which similar decisions are made in higher organisms.

Other Regulatory Effectors of Initiation

Several other regulatory proteins play subsidiary roles in the initiation of sporulation. Most of these have additional functions contributing to the control of various other responses, such as the development of genetic competence and the secretion of extracellular degradative enzymes, that are characteristic of the postexponential phase of growth.

***abrB*.** The *abrB* gene was originally identified by mutations that relieved some of the pleiotropic effects of mutations in *spo0A* (107, 108, 136, 137, 296). It is now known that the *abrB* gene encodes a DNA-binding protein (95, 279) that acts as a negative regulator of various genes that begin to be expressed in the postexponential phase (sometimes termed the transition state [217]). Among the genes controlled by AbrB are at least three sporulation genes, *spo0E* (216), *spo0H* (307, 327), and *spoVG* (326); an antibiotic-synthetic gene, *tycA* (95, 231); and *abrB* itself (278). Direct binding to promoter DNA has been demonstrated for all of these genes except *spo0H*. The negative autoregulation of the *abrB* gene presumably serves to maintain a steady level of AbrB in vegetative cells. The *abrB* gene is also negatively regulated by Spo0A (217), with the result that its level of expression

falls in the postexponential phase, thereby releasing various genes from repression.

hpr. Like *abrB*, the *hpr* gene encodes a small DNA-binding protein that controls expression of a number of postexponential-phase genes, particularly those encoding extracellular proteases (215). It probably controls at least one gene required for sporulation, because overexpression of *hpr* results in the inhibition of sporulation (215).

spo0J. The *spo0J* gene was first identified by a *spo* mutation, *spo0J93*, which arrests sporulation before septation (124) and was later identified by a transposon insertion (240). Originally, a second mutation, *spo-87*, was also placed in the *spo0J* locus, but it is now known to lie in a separate locus, *spoIIIJ*, concerned with stage III of sporulation (68). *spo0J* is one of only two *spo0* loci (the other being *spo0K*) that can be bypassed by infection with the spore-converting phages PMB12 and SP10 (159, 257). Following infection, spore-converting phages somehow intervene in the control mechanisms of sporulation in such a way that they advance its onset. They benefit by having their genomes trapped inside a spore and hence take advantage of the spore's properties of dormancy and resistance. Since these phages bypass *spo0J* and *spo0K* mutations, they probably advance the onset of sporulation in wild-type cells by acting on the pathway in which Spo0J and Spo0K normally operate.

There is some evidence that Spo0J is concerned with the catabolite repression of sporulation (203). The *spo0J* gene encodes a small protein with what is probably a DNA-binding motif (203), so it probably works by modulating gene expression. However, like most of the *spo0* gene products, it is not clear where Spo0J acts in the complex pattern of interactions controlling initiation. It will be interesting to discover precisely how phages PMB12 and SP10 replace or bypass Spo0J function; they may act in different ways (257).

sin. The *sin* (sporulation inhibition) gene was first isolated on the basis that it inhibited sporulation when placed on a multicopy plasmid (98), suggesting that the protein can negatively regulate certain essential sporulation genes. Subsequently, gene disruption experiments indicated that the protein was needed for the expression of various genes involved with other stationary-phase events, such as competence and motility (98). Indeed, the gene was identified independently on the basis of mutations preventing flagellin production (called *flaD* [248]). The Sin protein has been shown to bind to specific DNA sequences in vitro (99). Among the genes it controls are the important stage II sporulation genes in the *spoIIA*, *spoIIE*, and *spoIIG* operons (182). This negative regulation could serve to delay the expression of these genes until just before septation. Alternatively, Sin may provide a fail-safe mechanism for reversing entry into sporulation if nutritional conditions suddenly improve (182). Clearly, the level of Sin activity in the cell could influence the decision between sporulation and other stationary-phase responses, but its precise role is not yet clear.

Morphological Changes in the Preseptational Cell: Formation of the Asymmetric Septum

Clearly, much is known about the changes in transcription at the onset of sporulation, although many of the details remain to be worked out. We must now consider how they bring about the morphological events that occur at this stage.

The first overt sign of sporulation is the formation of the asymmetric spore septum. This is a modified form of the cell division septum (120), and its formation requires several genes, *ftsA* (originally called *spoIIG* or *spoIIN* [11, 102, 314]), *ftsZ* (originally called *divIA* [11, 13, 103]), and probably *divIB* (113) (also called *dds* [12]), that are involved in normal cell division. It seems likely that asymmetric septation is preceded by the inhibition of a septum destined to be formed in a central position, because mutations in some *spo0* genes (*spo0A*, *spo0K*, and possibly others [61, 221]) produce a phenotype in which continued division gives rise to cells that are approximately half the length of sporulating cells (Fig. 1). In contrast, *spo0H* mutations appear to be able to block this additional central septum, though they cannot make asymmetric septa (102).

The *spo-87* mutation was originally also placed in this category (124), but it is now known to be blocked at stage III rather than stage 0 (68, 273). Unfortunately, the cell length phenotypes of most of the well-characterized *spo0* mutants have not been described.

***spoIIE* and the modified spore septum.** Since the spore septum is probably produced by a modification of the process used in dividing vegetative cells, some (perhaps most) of the proteins needed for septum formation are likely to be common to both vegetative and spore septa. *spoIIE* mutants, however, seem to be affected in sporulation only. They produce asymmetric septa that are much thicker than normal, having a vegetative-like appearance (129, 221). It thus seems likely that the product of the *spoIIE* gene (317) somehow interacts with the general septum-forming apparatus to generate the modified spore septum. The time of *spoIIE* expression (just before septation [Table 1]) and the likely membrane location of its product (317) are in accordance with this notion.

Septum positioning. The mechanism responsible for the asymmetric positioning of the spore septum is as yet unknown, but this is not surprising in the light of our ignorance of the mechanism responsible for the central positioning of the vegetative septum. Nevertheless, the dispensability of the spore septum makes this an attractive area for future study. Prespores superficially resemble the minicells made by certain division mutants of both *B. subtilis* (229) and *E. coli* (2). However, during sporulation the small compartment that is partitioned off invariably contains a chromosome, whereas minicells are usually anucleate.

A tentative model explaining some aspects of the generation of asymmetry is discussed below in dealing with transcriptional regulation at the time of septation.

ESTABLISHMENT OF DIFFERENTIAL GENE EXPRESSION

It is clear from the above discussion of the results summarized in Tables 1 and 2 that soon after the completion of the spore septum, many genes are turned on specifically in the mother cell compartment and at least two are turned on in the prespore. Transcription in these two compartments seems to be mediated by different sigma factors, σ^E and σ^F (57, 71, 72, 183), but the genes encoding these two key transcription factors, *spoIIGB* and *spoIIAC*, are expressed in the preseptational cell (101, 209). How are the activities of σ^E and σ^F restricted to a single compartment after septation? Although the answer to this question is not yet complete, progress is beginning to be made: regulatory mechanisms capable of controlling the activities of σ^E and σ^F have been uncovered and are partially understood (Fig. 4 and 5).

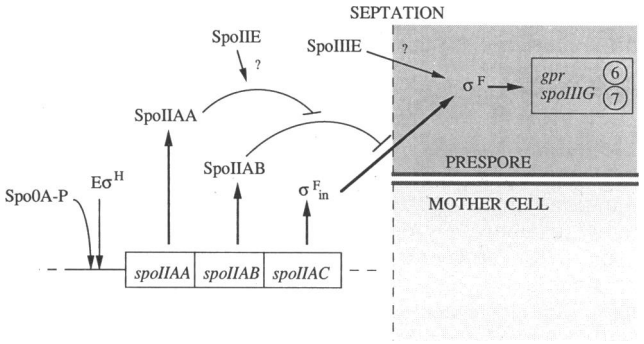

FIG. 4. Regulation of σ^F activity. σ^F is encoded by the third gene in the *spoIIA* operon (70, 87, 270, 283), which is expressed before septation (i.e., in the preseptational cell [unshaded]) (101, 209) as a result of transcription by $E\sigma^H$ (309) in conjunction with the phosphorylated form of Spo0A (Spo0A-P [218]). The other products of the operon, SpoIIAA and SpoIIAB, control σ^F activity, probably through the negative regulatory cascade implied by the two lines ending in bars (183, 211, 247). σ^F is thus rendered inactive in the preseptational cell. After septation, σ^F activity is released specifically in the prespore compartment, where it activates transcription of at least two genes, *gpr* (183, 284) and *spoIIIG* (211, 247, 283). The release of σ^F activity requires the products of the *spoIIE* (183) and *spoIIIE* (88) genes. The precise points at which these proteins intervene are unknown, but the SpoIIE proteins probably act at a relatively early step, perhaps the formation of the spore septum (129, 273), and SpoIIIE proteins act rather later. The numbers 6 and 7 refer to the dependence classes in which *gpr* and *spoIIIG* are placed in Table 2. These genes are placed in distinct categories because *spoIIIG* transcription is blocked by mutations in *spoIIG* (encoding σ^E in the mother cell), whereas *gpr* transcription is independent of *spoIIG* (209, 210).

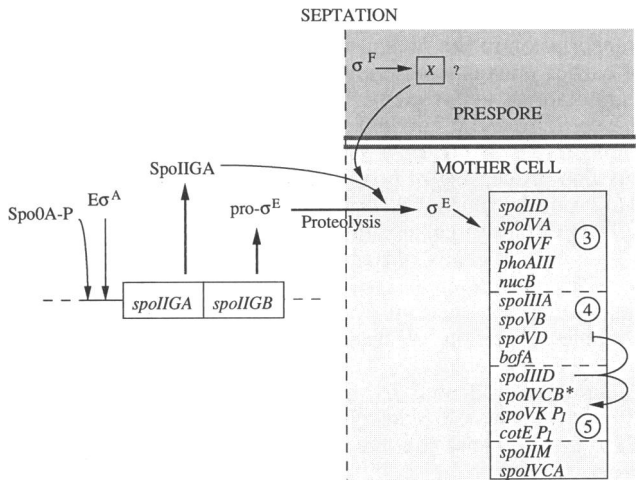

FIG. 5. Regulation of σ^E activity. σ^E is synthesized as an inactive precursor, pro-σ^E (168), which is encoded by the second gene in the *spoIIG* operon (148, 273, 274, 294). This operon is transcribed in the preseptational cell by $E\sigma^A$ in conjunction with the phosphorylated form of Spo0A (Spo0A-P) (156, 244, 245). The first gene in the operon, *spoIIGA*, probably encodes the protease required for formation of mature σ^E (148, 273). σ^E activity seems to be restricted to the mother cell after septation (57, 72); the simplest explanation is that processing occurs only in this compartment. Both processing (145) and σ^E activity (72) are abolished by certain mutations in the *spoIIA* operon, suggesting that a gene product synthesized specifically in the prespore compartment as a result of transcription by σ^F (*X*) acts vectorially across the spore septum to trigger processing of pro-σ^E only in the mother cell (178, 183). *spoIIE* mutations, which block the appearance of σ^F activity (183) (Fig. 4), could act additionally to prevent transmission through the septum of the putative signal from σ^F (not shown here). σ^E is known to direct the transcription of a number of genes, as shown (Table 1). The positive and negative regulatory effects of one of the genes in the σ^E regulon, *spoIIID*, are also shown (Tables 1 and 2); the effects of *spoIIID* mutations on expression of *spoIIM* and *spoIVCA* have not been reported. The numbers to the right refer to the classes of gene expression defined in Table 2. *spoIVCB* is starred because its promoter is also transcribed by σ^K during the later stages of sporulation, and it was therefore placed in a separate dependence class (class 9) in Table 2.

Control of σ^E and σ^F Activities

Within the family of sigma factors, σ^E is unusual in being synthesized as an inactive precursor with an N-terminal 29-amino-acid prosequence (147, 168). Pro-σ^E is encoded by the second of the two genes that make up the *spoIIG* operon (155, 294). The first gene in the operon, *spoIIGA*, probably encodes the protease needed to activate σ^E (148, 273) (Fig. 5).

In a similar way, the *spoIIA* operon encodes both σ^F and proteins that regulate its activity. σ^F is encoded by the third gene of the operon, *spoIIAC* (70, 283). The product of the second gene, *spoIIAB*, seems to be a negative regulator of σ^F activity, and its action is in turn antagonized by the product of the first gene, *spoIIAA* (37, 183, 211, 247) (Fig. 4). Thus, mutations in *spoIIAB* cause overexpression of σ^F-dependent genes, whereas *spoIIAA* mutations generally abolish σ^F activity. SpoIIAA probably acts through SpoIIAB because double mutants exhibit a *spoIIAB*-like phenotype (183, 247). The molecular basis for the inhibition of σ^F activity by SpoIIAB is not yet understood, but it does not seem to involve proteolysis in the manner of σ^E inhibition.

Role of the Spore Septum in Activation of σ^E and σ^F

The activities of both σ^E and σ^F seem to be dependent in some way on the formation of the specialized spore septum. Both sigma factor activities are abolished in the presence of *spoIIE* mutations (Table 2), which result in the formation of an abnormal septum containing excess wall material (see above), or when synthesis of the essential division initiation protein FtsZ is withheld (13, 185). However, penicillin, which inhibits septation at a relatively late stage (compared with the time at which FtsZ acts), has only a slight effect on σ^E activity (146).

The notion of coupling the activation of gene expression to the formation of a morphological structure (168, 273) is attractive because it would provide an effective means both of coordinating the genetic program with morphological development and of establishing temporal control over prespore-specific and mother cell-specific gene expression. However, as discussed below, it is possible that the early stages of sporulation involve a series of intercellular signals, which play crucial roles in ensuring that the two sister cells achieve their different determined states. If so, it would be expected that mutations or treatments that prevented intercellular communication across the septum would interfere with subsequent gene expression.

Dependence of σ^E Activity on σ^F

An appraisal of the collections of genes known to be controlled by σ^E and σ^F leads to the inescapable conclusion that they act more or less concurrently after septation, σ^E

directing transcription in the mother cell and σ^F directing transcription in the prespore (72). Paradoxically, the results of earlier epistasis experiments had suggested that the two sigma factors act sequentially. Thus, nonsense mutations in the *spoIIAC* gene encoding σ^F [*spoIIAC*(N) mutants] were found to block not only prespore-specific gene expression but also processing of pro-σ^E (72, 145). However, missense mutations in *spoIIAC* [*spoIIAC*(P) mutants [129]) behaved quite differently; usually they abolished the transcription of σ^F-dependent genes but not pro-σ^E processing (72). Moreover, mutations in the *spoIIIE* gene can also abolish prespore-specific gene expression by σ^F without having a detectable effect on σ^E-dependent gene expression in the mother cell (Table 2). (It should be noted that the *spoIIIE* effect is complicated by a strange chromosomal position effect, the physiological relevance of which is not yet clear [280, 281].) There are two ways to explain these results: processing of pro-σ^E could require σ^F but not necessarily in a form that is transcriptionally active, or an exceedingly small amount of a σ^F-dependent gene product could be sufficient to trigger almost wholesale processing of pro-σ^E. On the basis of the dependence of pro-σ^E processing on σ^F activity, Losick and colleagues (178, 183) have suggested the following sequence of events. Soon after septation, σ^F activity is released in the prespore by an as yet undefined mechanism presumably involving the SpoIIAA and SpoIIAB proteins. This results in the transcription of a gene whose product acts vectorially across the spore septum to trigger pro-σ^E processing in the mother cell (Fig. 5). This model is very attractive because it explains the mother cell specificity of σ^E, although it does not address the localization of σ^F activity.

The establishment of differential gene expression by the segregation of σ^E and σ^F activities after septation has been reviewed in more detail elsewhere (72, 178).

σ^F and Determination of Cell Fate

From the arguments presented above, it seems that the prespore-specific activation of σ^F could be crucial in determining the developmental fates of both prespore and mother cell. Clearly, the initiation of the prespore and mother cell programs of gene expression in the correct cellular compartments is crucial to the success of sporulation. Triggering of mother cell development is inherently risky because it culminates in cell lysis, which is lethal for the whole organism unless it occurs in conjunction with a fully committed prespore program in the adjoining cell. It is equally important that the prespore program be undertaken only with the active participation of the fully determined mother cell compartment. An exceedingly sensitive but accurate signal from prespore to mother cell after septation, such as the one postulated to lead from active σ^F to pro-σ^E processing (178, 183) (Fig. 5), could play a crucial role in the determination of cell fate during sporulation.

STAGE II TO III

Soon after septation, the chromosomes of the prespore and mother cell embark on different programs of gene expression as a result of the segregation of the activities of the key transcription factors, σ^E and σ^F. The next part of the review focuses on the general functions of these two transcription factors and the ways in which they initiate the differentiation of prespore and mother cell. We shall see that there are many more genes known to be transcribed by Eσ^E in the mother cell than by Eσ^F in the prespore.

σ^E and Determination of Cell Fate

Mutations in genes that allow progress to the septation stage but then prevent synthesis of mature σ^E, such as mutations in the *spoIIGB* gene itself, lead to a phenotype known as abortively disporic, in which a sporelike septum forms at both ends of the preseptational cell (Fig. 1). Both of the small polar compartments contain a chromosome, leaving an anucleate central compartment (250). The timing of septum formation in such mutants has recently been measured in two different ways: indirectly, by observing nucleoid segregation to the poles of the cell (210), and more directly, by electron microscopy of thin sections (67). The results indicate that one septum is usually completed before the other is started. This suggests that in wild-type cells the asymmetric septum is formed in at least two stages. The first involves the relocation of the sites of septum formation to the poles of the cell. It appears that there are potential septation sites near each pole at this stage. Asymmetry is then generated by a mechanism that somehow allows initiation to occur at one of these sites before the other. Perhaps this could be due to a limiting amount of some essential component such as the FtsZ septum initiation protein (15, 303). It does not seem to involve a choice based on DNA strand asymmetry (79). The second stage involves a block to the second potential division site that is triggered in some way by the completion of the first septum. Since the abortively disporic phenotype correlates with the absence of σ^E (129) and since σ^E synthesis is thought to be coupled to the formation of the spore septum (see above), it seems likely that σ^E directs the synthesis of an inhibitor of septation (210, 271). Mutations in the gene encoding such an inhibitor would be expected to give an abortively disporic phenotype without disrupting σ^E activity, but no such mutations have yet been found.

In addition to their effect on cell morphology, *spoIIG* mutations cause an increase in the initial rate of expression of the *gpr* gene, a prespore-specific gene transcribed soon after septation by Eσ^F (see below). This may be significant because it raises the possibility that in abortively disporic cells σ^F becomes active in both small compartments. If this is true, σ^E also has a role in cell determination by preventing the large compartment from entering the prespore developmental program (210). Whether this occurs indirectly, as a consequence of preventing the formation of the second small compartment, or by a more direct effect on σ^F activity is not yet clear.

σ^E and Engulfment

Following the completion of the spore septum and the establishment of σ^E and σ^F in the mother cell and prespore, respectively, development proceeds by the engulfment of the prespore within the cytoplasm of the mother cell. This remarkable process is unique to bacterial endospore formation, although it superficially resembles phagocytosis in eukaryotic cells. The functions required for engulfment are poorly characterized, and the physical process has not been investigated in any detail. It might involve the action of a specific protein that mediates membrane fusion. The membrane movements could be driven by hydrostatic pressure, for example by changing the relative osmolarities of the two cells, given that the total volume of the cell pair is fixed by

their common cell wall. There is also a substantial increase in the total membrane surface area during progression from stage II to stage III, so engulfment may require an increase in phospholipid synthesis or maintenance of the rate of phospholipid synthesis coupled with a reduction in cell wall elongation.

In contrast to the lack of progress in understanding the biophysics of engulfment, the study of mutant phenotypes has been fruitful. It is now recognized that engulfment proceeds in several discrete steps, each requiring at least one specific gene product (129). Some of the components necessary for the earliest steps of engulfment are expressed before septation. Thus, *spoIIB spoVG* double mutants fail to progress beyond the completion of the spore septum (stage IIi [Fig. 1]) (184). These genes apparently encode partially redundant functions involved in the earliest stage of engulfment (184). At least one σ^E-dependent gene must also be required for the initiation of engulfment because *spoIIG* mutants (which eliminate σ^E) not only fail to block the second polar septum but also fail to show any further development of the first septum beyond its completion (129).

Engulfment appears to begin with PG hydrolysis near the central part of the septal disc. This is accompanied by bulging of the two now closely opposed membranes into the mother cell cytoplasm. Mutations in the σ^E-dependent *spoIID* gene cause a morphological block at this intermediate stage (stage IIii [Fig. 1]). Since these mutants do not complete the hydrolysis of the septal PG, it seems that discrete functions may be needed for the onset and the completion of septal PG hydrolysis. Completion of septal PG hydrolysis allows the edges of the septum to begin to migrate toward the proximal pole of the cell (stage IIiii). Since certain mutations in the gene encoding σ^F can develop as far as stage IIiii (those which allow pro-σ^E processing [see above and reference 129]), it seems likely that at least one other function necessary for the transition from stage IIi to IIii is dependent on a gene controlled by σ^E. Finally, the edges of the septal membranes meet at the pole of the cell and fuse, completing the engulfment of the prespore within the mother cell cytoplasm (stage III). Since this final step is prevented by the *spoIIAC*(P) mutations (129), it is possible that a σ^F-dependent gene is necessary for the completion of engulfment, but it is also possible that the effect on engulfment is due to a reduction in the concentration of a σ^E-dependent gene product in these mutants. It should be noted that mutations in *spoIIIE*, which block prespore gene expression but have no apparent effect on σ^E-dependent gene expression (Table 1), allow engulfment to be completed (221).

A number of other classes of genes begin to be activated during stage II to III of sporulation. Most of these will be discussed below in sections on specific events associated with the later stages of sporulation.

σ^E-Dependent Extracellular Enzyme Production

The production of extracellular degradative enzymes has already been mentioned during the discussion of the initiation of sporulation. The secreted proteins presumably play a scavenging role, increasing the availability of nutrients under starvation conditions. Several such enzymes, particularly amylases and proteases, are synthesized soon after the onset of sporulation. Most of these enzymes are also synthesized under stationary-phase conditions that do not favour sporulation. However, at least two different extracellular enzymes are synthesized at an intermediate stage of sporulation, as part of the σ^E regulon.

APase. Several distinct genes encode proteins with alkaline phosphatase (APase) activity (16, 125, 150). Some of these are required for the production of this enzyme in vegetative cells starved for phosphate, and there are distinct forms differing in their localization, e.g., cell bound or secreted. During sporulation, one or more proteins with APase activity appear during stage II (304) and the onset of APase synthesis coincides with the activation of several σ^E-dependent genes (130, 209). The activity also behaves, in the presence of a range of *spo* mutations, as if it has a simple σ^E-dependent promoter (4, 72, 74, 221, 301). About 50% of the sporulation APase is now known to be produced by the *phoAIII* gene, which has been cloned and characterized (16). This gene has two promoters: P_v is active in vegetative cells starved for phosphate; P_s is specific to sporulation. Promoter P_s bears some resemblance to the σ^E consensus. The gene responsible for the remaining sporulation-specific APase has not yet been identified.

DNase. Akrigg (3) has described a 12-kDa extracellular DNase that has a very similar dependence pattern to that of APase, although its expression begins somewhat later than that of APase (4). The delay in appearance of the activity could be due to the action of a DNase inhibitor (225). A gene capable of encoding a nuclease of about the appropriate size, *nucB*, has recently been cloned (300). Immediately upstream of the putative translation initiation site are sequences very similar to those of other σ^E-dependent genes.

The fact that the mother cell seems to be specifically concerned with the secretion of various enzymes emphasizes the functional differentiation of the two cells.

σ^E Regulon: Transcriptional Modulation by the SpoIIID Protein

Many of the genes of the σ^E regulon that are active soon after septation seem to be subjected to an additional level of transcriptional regulation that may serve to fine-tune their timing, their levels of expression, or both, in accordance with the requirements for their products. The *spoIIID* gene encodes a small (11-kDa) DNA-binding protein with a helix-turn-helix motif (162, 164, 268). As summarized in Table 2, SpoIIID can exert positive or negative control over the expression of various genes in the σ^E regulon. Although *spoIIID* is undoubtedly expressed at least initially by $E\sigma^E$ and SpoIIID controls the expression of many σ^E-dependent genes, its effects on transcription in vitro were first observed in conjunction with $E\sigma^K$ (162). The possibility that SpoIIID continues to act in the later part of mother cell development is discussed below.

We have previously suggested that SpoIIID may act as a simple time switch, dividing the genes of the σ^E regulon into separate temporal classes (64, 130). Because the *spoIIID* gene has a partial requirement for its own product (164, 268), full expression of the *spoIIID* gene is delayed by 10 or 20 min compared with that of other σ^E-dependent genes (Table 1). Therefore, other genes that require SpoIIID (e.g., *spoVK*) show a similarly delayed onset of expression. Conversely, genes repressed by SpoIIID begin to be transcribed as soon as σ^E appears but their expression is transient because they are shut down when enough of the SpoIIID product has accumulated. This simple mechanism results in the division of the σ^E regulon into three distinct temporal classes, as shown in Table 2. Class 3 genes, exemplified by *spoIID*, begin to be expressed as soon as σ^E activity appears. Class

4 genes, e.g., *spoIIIA*, become active at the same time, but their expression is relatively transient. Class 5 genes, e.g., *spoVK*, are delayed in their expression for a short period until sufficient SpoIIID has accumulated. Thus, the main role of SpoIIID may be to optimize the temporal expression of individual genes in the σ^E regulon (and probably the σ^K regulon [see below]).

General Role of σ^F

Soon after the spore septum is completed, σ^F, formed in the preseptational cell, becomes active specifically in the prespore, but surprisingly little is known about the genes that it controls. In fact, only two genes are known to be transcribed by $E\sigma^F$ in vivo, *gpr* and *spoIIIG* (183, 211, 247, 280, 284). This contrasts with the early phase of mother cell development, in which more than 10 genes transcribed by $E\sigma^E$ have now been characterized (see above; Table 1). This may reflect the different developmental fates of the two cells. The prespore represents a germ line cell: eventually it must be capable of dedifferentiation into a vegetative cell via the processes of germination and outgrowth. This requirement may severely limit its degree of specialization. In contrast, the mother cell has no such restraints: it exhibits terminal differentiation and could, in principle, devote all of its resources to the maturation of the spore. It may thus be that σ^F controls genes involved in a general adaptation for the stationary phase. Circumstantial evidence supports this notion. The *spoIIAC* gene encoding σ^F is extremely toxic in *E. coli* (318, 319). This toxicity may be due to the inappropriate expression, during growth, of genes that are normally expressed only during the stationary phase of *E. coli*: preliminary experiments show that *E. coli* contains an RNA polymerase that can direct transcription from the *spoIIIG* promoter (78). Conservation of a regulatory system controlling a general response to stationary phase would make sense from the evolutionary point of view because the problems associated with starvation are faced by all bacterial cells and surely predated the evolution of spore formation. Clearly, this suggestion should be tested by the isolation and characterization of more σ^F-dependent genes. The functions and regulation of the two known σ^F-dependent genes, *gpr* and *spoIIIG*, will be discussed in detail below.

σ^G AND TRANSITION TO THE LATE PHASE OF PRESPORE DEVELOPMENT

About 2 h after the initiation of sporulation, engulfment of the prespore is complete and synthesis of σ^G, the product of the *spoIIIG* gene, begins. This results in the activation of a battery of genes, the σ^G regulon, whose products effect numerous changes in the physiological and biochemical properties of the spore. The formation of active σ^G seems to be regulated in at least three different ways.

Transcriptional Regulation of *spoIIIG*

The *spoIIIG* gene lies in a curious position immediately downstream of the *spoIIG* operon, which encodes the early mother cell sigma factor, σ^E (151, 192). Indeed, transcription from the *spoIIG* promoter, which is active in the preseptational cell, reads through into the *spoIIIG* gene (192, 280). However, it is not clear whether this transcription serves any important purpose, because transcription from this upstream promoter is not necessary for efficient sporulation (155), and it appears that secondary structure in the mRNA prevents translation of *spoIIIG* from mRNA molecules initiated at the upstream start site (192, 280).

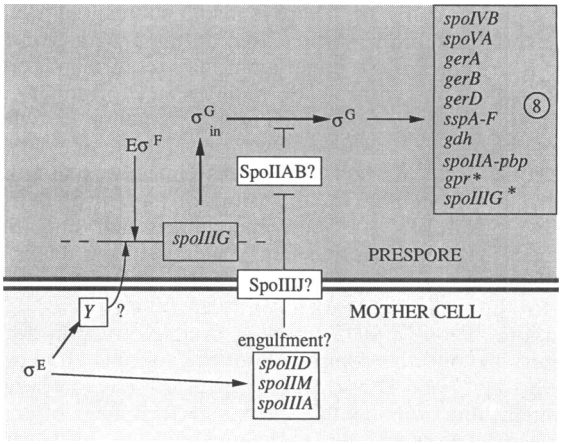

FIG. 6. Regulation of σ^G synthesis and activity. Transcription of the *spoIIIG* gene occurs predominantly from a promoter recognized by $E\sigma^F$ and is thus rendered prespore specific (183, 209, 211, 247, 283). The strong effects of mutations in the *spoIIG* gene on transcription of *spoIIIG* suggest the existence of an additional form of transcriptional regulation that is somehow controlled by events occurring in the mother cell (209). This is depicted by an as yet unknown gene, *Y*, transcribed by $E\sigma^E$ in the mother cell, which is shown acting across the spore septum to control *spoIIIG* transcription. σ^G also seems to be regulated at a posttranscriptional level (178, 272). Thus, the gene is transcribed, but σ^G activity is blocked or reduced, in the presence of several different classes of mutation. Some of these mutations lie in mother cell-specific genes controlled by σ^E; at least one lies in the vegetatively expressed gene *spoIIIJ* (Table 1). The signal apparently leading from mother cell to prespore (perhaps via SpoIIIJ) could provide an important means of coordinating gene expression in the two cells, and/or it could delay the onset of σ^G activity until some structural feature of the developing organism is completed, for example, prespore engulfment (178, 272). In either case, the delay in σ^G activity suggests the existence of an inhibitor, and the SpoIIAB protein (unshaded, as it is synthesized in the preseptational cell) is a good candidate for this factor (90, 227, 272). The appearance of active σ^G leads to the expression of a number of prespore-specific genes, as shown. The *gpr* and *spoIIIG* genes are starred because their promoters are also recognized by $E\sigma^F$ in vitro and in vivo (183, 211, 247, 283, 284). The number 8 to the right indicates that these genes (other than *gpr* and *spoIIIG*) were denoted class 8 in Table 2.

The major transcription start site for *spoIIIG* can be used by σ^F both in vitro and in vivo (211, 247, 280) and gives rise to mRNA that is translated to give σ^G (Fig. 6). The promoter is also recognized by RNA polymerase containing σ^G, the product of its own gene, but with lower relative affinity (280). Transcription from this promoter begins at about 120 min, after prespore engulfment is complete (88, 209). This time coincides with strong transcription of the other known σ^F-dependent gene, *gpr*, but is about 40 min later than an early weak phase of *gpr* expression, which is presumed to represent the onset of σ^F activity in the prespore (183, 209). The *spoIIIG* promoter also differs from the *gpr* promoter with respect to the effects of *spoIIG* mutations (209). These enhance the early phase of *gpr* expression (see above) but abolish transcription of *spoIIIG* (Table 2). It thus seems that *spoIIIG* is subject to at least one level of transcriptional regulation that does not act on *gpr*. The effect of *spoIIG* mutations is particularly interesting because the *spoIIG* operon is responsible for the synthesis of σ^E, which operates

specifically in the mother cell. This again implies the existence of an intercompartmental signal coordinating development in the two cells (Fig. 6).

Control of σ^G Activity

The ability of both $E\sigma^F$ and $E\sigma^G$ to transcribe from the *gpr* and the *spoIIIG* promoters obscures our understanding of their regulation. Although somewhat conflicting results have been reported, mutations in the *spoIIIG* gene, abolishing σ^G synthesis, probably reduce expression of both *gpr* and *spoIIIG* by about twofold (88, 151, 209, 281). The straightforward explanation for the reduction in transcription would be that it reflects loss of the contribution to transcription normally made by $E\sigma^G$. However, there are reasons for doubting this interpretation. The effect of *spoIIIG* mutations on transcription of these genes is apparent at 120 min, but the activation of strictly σ^G-dependent genes, such as *sspB* and *spoVA*, does not begin until about 150 min (i.e., 30 min after *spoIIIG* transcription begins), suggesting that σ^G activity does not appear until this time (209). In other words, the *spoIIIG* mutation appears to have an effect on the transcription of its gene 30 min before σ^G activity would normally appear.

One way to reconcile these observations would be to suppose that σ^F and σ^G compete for an inhibitor: the product of the *spoIIAB* gene is known to be capable of inhibiting both proteins (227) (see above). Until 120 min, SpoIIAB would be inhibiting only σ^F. The onset of σ^G synthesis could then enhance transcription of σ^F-dependent genes, such as *gpr* and *spoIIIG* itself, by competing for the inhibitor of σ^F rather than by acting directly as a transcription factor. Such an effect would not be surprising given the closely related primary sequences of σ^F and σ^G (66). Thus, σ^F could be primarily responsible for transcription of *spoIIIG*.

A second reason for supposing that σ^G might be regulated at a posttranscriptional level arises from the observation that mutations in several genes block or severely reduce the expression of genes in the σ^G regulon but have relatively mild effects on the transcription of *spoIIIG* itself (272) (Table 2). In terms of dependence interactions, these genes have very similar effects to mutations in *spoIIIG* itself, as if their main effect is to eliminate σ^G activity.

The genes in this class vary considerably in terms of their regulation and the phenotypic effects of mutations (Table 1; Fig. 1). Mutations in *spoIIB* (together with *spoVG*; see above), *spoIID*, and *spoIIM* block sporulation prior to engulfment (36, 184, 240, 317): mutations in the other genes, *spoIIIA* and *spoIIIJ*, allow engulfment to be completed (stage III) but permit no further progress (68, 221, 273). *spoIIIJ* is expressed predominantly in vegetative cells. *spoIIB* and *spoVG* are members of the σ^H regulon and thus are expressed in the preseptational cell. *spoIID*, *spoIIM*, and *spoIIIA* are probably expressed specifically in the mother cell, being members of the σ^E regulon. All the genes have been sequenced, at least in part. The predicted *spoIIB* and *spoIID* proteins share sequence similarity to genes involved in PG metabolism (167, 171, 184), which would be consistent with their apparent roles in the early stages of engulfment. The *spoIIM* product is extremely hydrophobic, suggesting that it is an integral membrane protein, but it exhibits no significant similarities to known proteins in the sequence data bases (317). The predicted protein product of the *spoIIIJ* gene is also rather basic and hydrophobic, and it is probably a lipoprotein (68). This could result in some of the protein's being positioned between the spore membranes after engulfment, where, in principle, it could influence transactions between the two cells. The *spoIIIA* locus comprises a large operon containing at least four genes (130). DNA sequence analysis indicates that some of the products may be membrane associated, and the first gene has consensus-binding sites for nucleotides such as ATP (130, 131).

There are several ways to explain the effects of these mutations. On the basis of the requirement for several σ^E-dependent genes, it appears that the onset of the late phase of prespore development is coupled in some way to events in the mother cell, not only (as described above) at the level of *spoIIIG* transcription but also at the level of σ^G activity. Perhaps, in extension of the above discussion, this occurs via the SpoIIAB protein, as shown in Fig. 6. The effects of the mutations on morphological development have prompted Stragier (272) to suggest that the activation of σ^G is mechanistically coupled to the completion of engulfment in a manner reminiscent of the proposed role for the spore septum in the processing of σ^E (see above). The completion of engulfment certainly has a variety of effects on the physiology of the prespore, since its cytoplasmic membrane no longer communicates directly with the external medium. Mutations blocking engulfment of the forespore would obviously prevent these dramatic changes from occurring. If the activation of σ^G were coupled to one of these changes, the effects of the mutations preventing engulfment would be explained. Given the topological position of the prespore inside the mother cell cytoplasm, it seems likely that the prespore may require specific biochemical channels to maintain its biosynthetic capability during the late stages of development, especially under the circumstances of starvation. A less specific explanation for the effects of mutations in genes such as *spoIIIA* and *spoIIIJ* would be that their products are required to maintain the general metabolic activity of the prespore. Loss of such functions might lead to a general reduction of prespore-specific gene expression (128). Indeed, all of the mutations in this class affect the morphological development of the cell, and some of the mutants exhibit abnormal morphology by the time *spoIIIG* transcription normally begins. Thus, relatively nonspecific explanations for the effects on gene expression should not be ruled out. Nevertheless, it seems possible that another specific intercellular interaction couples the activation of σ^G in the prespore to the mother cell program of gene expression.

Later Temporal Class of Prespore Gene Expression?

Most of the well-characterized prespore-specific genes are turned on simultaneously at about $t_{2.5}$ as a result of the onset of σ^G activity (Table 1). However, three genes may belong to a later class of gene expression. The *0.3 kb* gene (now called *sspF* [see below]) is expressed about 1 h later than other known σ^G-dependent genes (208). The sequence upstream of the *sspF* gene resembles those of other σ^G-dependent promoters only loosely. However, expression of *sspF* is induced, albeit after a short delay, by synthesis of σ^G in vegetative cells (208), suggesting that it could be transcribed by $E\sigma^G$ in conjunction with some other factor.

Recent results suggest that a gene lying immediately upstream of the *spoIIA* operon, which appears to encode a low-molecular-weight penicillin-binding protein PBP (*dacF* [see below]) may be regulated in a similar way to the *sspF* gene (310). Its putative promoter sequences closely resemble those of *sspF*, and it is expressed relatively late in sporulation (310). The *spoIVB* gene (see below) also appears to

exhibit delayed prespore-specific transcription, but its promoter sequences are rather similar to those of other σ^G-dependent genes. No factor capable of modulating the transcription of prespore-specific genes has been found so far, so the molecular basis of these effects is unknown.

FUNCTIONS OF GENES IN THE σ^G REGULON

More than a dozen genes belonging to the σ^G regulon have now been characterized. What are their functions, and how do they contribute to the biochemical and morphological maturation of the prespore?

ssp Genes: Resistance to UV and Storage of Amino Acids

At least six genes controlled by σ^G encode SASPs found in the spore core. The structure, synthesis, genetics, function, and degradation of the SASPs have been comprehensively reviewed by Setlow (254, 255). Two distinct classes of SASP have been distinguished. The smaller α/β-type SASPs, form a multigene family with a high degree of sequence conservation both between different genes in the same organism and between organisms. In *B. subtilis*, four genes encoding α/β type SASPs have been cloned (*sspA* through *sspD*). They are monocistronic and map at different chromosomal locations (35). The γ-type SASPs are slightly larger (molecular weight 8,000 to 11,000 compared with 5,000 to 7,000 for the α/β class) and are invariably encoded by a single gene (*sspE* in *B. subtilis*). Recently it has been recognized that the *0.3 kb* gene (see above), originally cloned on the basis of its abundant long-lived mRNA (265), encodes a protein closely related to the SASPs (254). It is proposed to redesignate this gene *sspF*, although its functional relationship to the other *ssp* genes is not yet clear. All of the SASPs have a short conserved sequence recognized by a specific protease encoded by the *gpr* gene. (Strictly, the *gpr* gene could be regarded as belonging to the σ^F regulon, and its promoter has already been discussed above in this context.) The synthesis of this protein poses an interesting problem because its action must be regulated so that degradation of the SASPs begins only after germination. The protease seems to be synthesized as an inactive precursor with a molecular mass of 46 kDa, which is cleaved to a 41-kDa form during later sporulation and to a 40-kDa form during germination (110, 174). Both of the lower-molecular-mass forms have protease activity in vitro, so it is not yet clear how premature destruction of the SASPs is avoided. Cleavage of the SASPs by this protease during spore germination initiates a rapid and complete degradation of the proteins, supplying a ready source of amino acids for protein synthesis during outgrowth. A major function of the SASPs is thus probably to act as amino acid storage proteins (254).

No function other than amino acid storage has yet been found for the γ-type SASPs, but the α/β type SASPs are associated with the spore nucleoid (91). Their binding to DNA (196, 205, 249) causes an increase in negative superhelicity (205, 206) and a B- to A-form transition (196) and contributes to the resistance of spores to UV irradiation and possibly to heat (189, 190, 251).

As discussed above, the *ssp* genes are all transcribed by the σ^G form of RNA polymerase, but additional levels of regulation are likely (254). Increasing the gene dosage of an α/β-type *ssp* gene results in increased production of both its mRNA and its gene product, with a corresponding decrease in the mRNA and protein levels of the other major SASPs, so that the total level of α/β-type SASPs remains more or less fixed. This suggests feedback regulation of transcription, perhaps mediated by the binding of the α/β-type SASPs to DNA. Increased dosage of neither an α- nor a β-type *ssp* gene had an effect on the γ-type SASP gene, *sspE*. Increasing the dosage of the *sspE* gene, in contrast, caused increased accumulation of mRNA but no increase in the protein level. This result suggests feedback regulation at the level of translation, but the molecular nature of these effects remain to be clarified.

Germination Genes of the Prespore

The properties of dormancy and resistance that typify bacterial endospores would be useless without some system for detecting when the environment is suitable for germination and the resumption of vegetative growth. At least three operons controlled by σ^G are essential for spore germination (see reference 198 for a recent review of germination genes). The properties of at least two of these operons suggest that they encode systems that receive an environmental signal and transduce this information to the mechanism controlling spore germination.

gerA. *gerA* is the best-characterized cluster of germination genes. It consists of an operon containing three genes, *gerAA*, *gerAB*, and *gerAC*, encoding products of 480, 364, and 373 amino acids, respectively. Mutations in any one of these genes cause a defect specifically in the germination response to L-alanine (237, 328). Each of the predicted gene products has sequences characteristic of proteins that are associated with membranes, suggesting that the three proteins form a membrane complex, presumably located in the inner prespore membrane (82, 328). The way in which the putative receptor complex senses L-alanine and transmits this information to the germination apparatus is unknown.

gerB. Nucleotide sequence analysis indicates that the *gerB* locus is closely related to *gerA*, encoding three genes of similar size and amino acid sequence (38). Mutations in *gerB* cause a defect in the germination response to a mixture of asparagine, glucose, fructose, and KCl (AGFK). Presumably, the GerB proteins act in much the same way as their counterparts in the *gerA* operon.

spoVAF. The distal part of the σ^G-dependent *spoVA* operon (see below) contains an open reading frame encoding a protein very similar to those encoded by the *gerAA* and *gerBA* genes (67). The reading frame actually overlaps the *spoVAE* gene by about 30 residues, so it is not clear whether this gene is part of the *spoVA* operon or is separately regulated. There do not seem to be homologs of the second and third genes of the *gerA* and *gerB* operons downstream. A mutation inactivating the *spoVAF* gene has been engineered (67), but no specific germination defect has yet been identified (152). The finding of three operons with related genes raises the possibility that the spore contains a collection of receptor complexes responsible for the responses to different germinants.

gerD. A germination gene affecting a later stage of germination, *gerD*, is also known to belong to the σ^G regulon (153). It encodes a single polypeptide that is essential for germination via the AGFK pathway and is required for the alanine response under certain conditions.

The functions of several other σ^G-dependent genes will be discussed in later sections.

CONTROL OF σ^K SYNTHESIS AND TRANSITION TO THE LATE PHASE OF MOTHER CELL DEVELOPMENT

The final stages of sporulation are controlled by a fourth sporulation-specific sigma factor, σ^K. Its synthesis results in activation of various genes that contribute to the final maturation and release of the spore, particularly those encoding the proteins that contribute to the synthesis and assembly of the protective layers of cortex and coat. Because these structures effectively seal off the prespore from the mother cell, the timing of σ^K synthesis is crucial. Thus, again, the regulation is complex, and one facet involves an intercellular interaction.

Regulation of σ^K Synthesis

Transcriptional regulation of *sigK*. σ^K synthesis is regulated at three distinct levels (Fig. 7). The first involves transcriptional regulation. The promoter of the *sigK* gene encoding σ^K is recognized initially by $E\sigma^E$, acting in conjunction with the SpoIIID positive regulator (see above). This ensures that transcription begins only after the appearance of σ^E and the accumulation of sufficient SpoIIID protein. However, this mode of transcription probably accounts for a relatively minor proportion of total *sigK* expression. The main phase of transcription is probably mediated by σ^K itself, again in conjunction with SpoIIID (162, 166). This is not as surprising as it might seem, because σ^E and σ^K, like their counterparts in the prespore, σ^F and σ^G, are closely related and have very similar recognition properties (reviewed in reference 66).

Site-specific recombination and role of *spoIVCA*. A second level of regulation that is exerted over σ^K synthesis involves a remarkable DNA rearrangement that takes place specifically in the mother cell (275) and is mediated by a site-specific recombinase encoded by the *spoIVCA* gene (165, 242). This brings together two partial coding sequences, originally defined by mutations designated *spoIVCB* and *spoIIIC*, which lie more than 40 kb apart on the vegetative chromosome. The intervening DNA, called *skin* (*sigK* intervening element [165]), which includes the *spoIVCA* recombinase gene, is excised as a circular DNA molecule (165). This event is developmentally regulated and occurs specifically in the mother cell compartment, at least partly because of the transcriptional regulation of *spoIVCA* (242). Although this extraordinary event could clearly exert very tight control over σ^K synthesis, it is probably not essential for sporulation, for two reasons. First, some closely related bacilli do not have the rearrangement (1). Second, strains genetically engineered to contain the rearranged gene, *sigK*, sporulate normally, and they are rendered independent of the recombinase gene and of both partial genes (165). Presumably the *skin* element is a relic of a defective prophage or transposable element. Because the rearrangement occurs specifically in the mother cell, it is not inherited by any descendent cells. Vegetative cells and prespores therefore contain the uninterrupted form of the *sigK* gene.

Pro-σ^K processing. In addition to the two levels of regulation already mentioned, a third level of regulation of σ^K synthesis has been uncovered. Like σ^E, its closely related predecessor in the developmental program, σ^K is synthesized as an inactive precursor, which is activated by proteolytic cleavage of an N-terminal pro sequence of 20 amino acids (40, 180). Processing is under complex control and is abolished by mutations in a range of genes with diverse functions (Table 2). Perhaps most interestingly, processing is blocked by mutations preventing synthesis of active σ^G, the prespore-specific sigma factor. In addition, several genes in the σ^E regulon are required for processing, directly or indirectly (180). The effects of mutations in some of these genes, such as *spoIID*, *spoIIM*, and *spoIIIA*, could be exerted, at least partially, through their effects on σ^G synthesis or activity (see above).

The requirement for σ^G in processing can be bypassed by mutations in two genetic loci called *bofA* and *bofB* (bypass of forespore [40]). *bofB* mutations lie in a σ^E-dependent, two-gene operon, *spoIVF* (44), and are missense or nonsense mutations near the C-terminal coding part of the *spoIVFA* gene. More extensive deletions in this gene confer a temper-

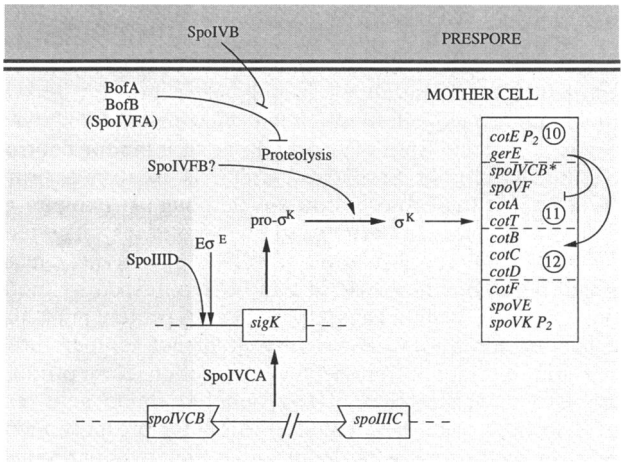

FIG. 7. Regulation of σ^K activity. Synthesis of σ^K is controlled at three distinct levels. The *sigK* gene is formed by site-specific recombination from two half genes, *spoIVCB* and *spoIIIC* (165, 275). The reaction is catalyzed by SpoIVCA (165, 242). The hybrid *sigK* gene is regulated at the transcriptional level, requiring the mother cell-specific sigma factor σ^E and the SpoIIID transcription factor (162, 166, 297). Finally, the product is, like σ^E, an inactive precursor requiring proteolysis (162, 180), which is probably catalyzed by the SpoIVFB protein (40, 44). Proteolysis is regulated by several proteins. The BofA and BofB proteins are negative regulators present on the mother cell side (40, 44, 134, 230). The SpoIVB protein provides at least part of a signal emanating from the prespore, which could coordinate gene expression in the prespore with that of the mother cell (43). Arbitrarily, this protein is shown antagonizing the inhibition exerted by BofA/BofB, but other forms of interaction are possible. No attempt has been made to show the possible membrane localization of several of the proteins, encoded by *bofA*, *bofB*, *spoIVB*, and *spoIVFB*, but this could clearly be significant. The formation of active σ^K leads to the activation of a number of genes that determine the late phase of mother cell development (Table 1). As in the case of σ^G, at least three of the genes controlled by σ^K are also transcribed by $E\sigma^E$, the preceding sigma factor in this compartment, indicated by asterisks (89, 162, 323). Positive and negative regulatory effects exerted by the GerE protein on various members of the σ^K regulon are also indicated (8, 50, 239, 322, 323). The numbers are again those used for the dependence classes in Table 2, except for *spoIVCB* (starred), which has complex regulation and was placed in a separate class (class 9). Again, the effect of *gerE* mutations on the expression of genes in the lower box has not been determined. The SpoIIID protein may also participate in the regulation of σ^K-dependent genes (162, 322) (see text).

ature-sensitive asporogenous phenotype, and at the permissive temperature they also cause a partial Bof phenotype. The second gene in the operon, *spoIVFB*, is essential for sporulation, and mutations in this gene prevent pro-σ^K processing. To explain these effects, Cutting et al. (44) have proposed that the SpoIVFA protein is an inhibitor controlling the activity of SpoIVFB, which is in turn a prime candidate for the pro-σ^K protease (although it exhibits no obvious sequence similarity to known proteases). The temperature-sensitive sporulation-defective phenotype caused by null mutations in *spoIVFA* can be suppressed by mutations in *spoIVFB* (44). Thus, the SpoIVFA protein must also be needed for SpoIVFB stability at higher temperatures, suggesting a direct protein-protein interaction. Both proteins have extensive stretches of hydrophobic amino acid residues, so they could be located in the outer prespore membrane, in accordance with the idea that they may participate in a signal transduction pathway leading from the prespore (40).

Mutations in the *bofA* locus also relieve the requirement for most genes other than *spoIVF* in processing (40). *bofA* encodes a small, relatively hydrophobic protein that also belongs to the σ^E regulon, but its precise role is not yet known (134, 230).

As mentioned above, most of the mutations preventing pro-σ^K processing could act via their effects on σ^G activity in the prespore, since they prevent the appearance of σ^G activity and σ^G is needed for processing. The *spoIVB* gene provides at least part of the link between events in the prespore and pro-σ^K processing. Mutations in *spoIVB* have very similar phenotypic effects to those in *spoIVF* or the components of the *sigK* gene. They are blocked in the synthesis of both cortex and coat (36, 43, 299). The predicted *spoIVB* product (299) has a molecular weight of about 46,000 and is highly basic, with an N-terminal hydrophobic domain that could act as a signal peptide. A cysteine residue near a possible cleavage site suggests that the protein could, like GerM and SpoIIIJ, provide a lipoprotein attachment. In this case, the SpoIVB protein would be located between the prespore membranes but attached to the inner membrane. Here it could conceivably interact with the SpoIVF and BofA proteins already implicated in the regulation of σ^K processing (see above). The precise function of *spoIVB* is not known, but it may not be dedicated simply to the production of σ^K in the mother cell because bypassing its role in processing, e.g., by introduction of a *bof* mutation, does not restore spore formation (43).

The regulatory pathway that couples σ^K synthesis in the mother cell to the activity of one or more genes in the prespore represents yet another intercellular interaction coordinating the development of the two cells. As mentioned above, expression of *spoIVB* seems to be delayed relative to that of most of the genes in the σ^G regulon (Table 1). This further delay could be particularly significant because it would contribute to the timing of mature σ^K synthesis in the mother cell.

Synthesis of σ^K results in the activation of a number of new genes in the mother cell, which bring about the formation of the major protective structures of cortex and coat. The main function of the processing apparatus could be to delay the synthesis of σ^K until most of the genes in the σ^G operon have become active and begun to make the products that carry out the final stages of maturation in the core of the spore.

DPA SYNTHESIS AND TRANSPORT

Function of DPA

Dipicolinic acid (DPA) is a small molecule that is found almost exclusively in bacterial endospores, where it accumulates to high concentrations. Its possible role in the resistance of spores to heat has been the subject of considerable debate. The best evidence in favor of such a role arises from the experiments of Balassa et al. (10) with a *spoVF* mutant, believed to be damaged in the gene encoding DPA synthetase. This enzyme catalyzes the single reaction necessary for the production of DPA from dihydrodipicolinic acid, an intermediate in the lysine biosynthetic pathway (9). The *dpa-1* mutation results in spores that appear to be wild type, but these have only about 10% of the wild-type content of DPA and are heat sensitive. However, heat resistance can be restored by addition of exogenous DPA, which is taken up by an active process requiring energy (10). On the other hand, there are reports of mutants that are almost devoid of DPA but that nevertheless show considerable heat resistance (see, e.g., reference 112). This has led to suggestions that DPA may be important in the maintenance of the dehydrated, resistant state of the spore protoplast (reviewed in references 100 and 104).

DPA Synthesis

Although DPA accumulates in the prespore, its synthesis appears to occur specifically in the mother cell at a relatively late stage of development (5). This system thus represents an interesting example of cooperation between the two differentiating cells. A biological assay for DPA synthesis, based on the restoration of heat resistance in a *spoVF* mutant (10), was used to monitor the effects of mutations in a range of *spo* genes (69). The results were mainly consistent with the expectation that DPA synthesis was dependent on σ^K. For example, synthesis was undetectable in a *spoIVC* mutant or in strains carrying mutations in genes such as *spoIVB* and *spoIVF*, which are needed for processing of pro-σ^K. However, surprisingly, mutations in certain other genes that prevent pro-σ^K processing, *spoIIIA*, *spoIIIE*, and *spoIIIJ*, albeit by acting at an earlier point in the dependence sequence, had no effect on DPA synthesis (69). Characterization of the cloned *spoVF* locus (50) indicates that it consists of on operon encoding two proteins, both of which are required for DPA synthesis. Neither predicted protein shows significant homology to known protein sequences in the standard data bases. As expected, the operon is transcribed from a σ^K-dependent promoter, and the dependence of expression of the operon on various *spo* genes is consistent with this interpretation. It thus seems that a small amount of transcription of the *spoVF* operon (as would occur in the presence of mutations that abolish pro-σ^K processing) provides sufficient DPA synthetase activity to give a positive result in the biological assay (50).

DPA Transport: Possible Roles for *spoVA* and *spoVK*

As stated above, DPA synthesis seems to occur in the mother cell but DPA accumulates to high concentrations in the prespore, apparently complexed with Ca^{2+} (202). Most probably, DPA is actively transported into the prespore and Ca^{2+} enters by facilitated diffusion (62). The entry of Ca^{2+} is probably enhanced by an uptake system in the mother cell (122).

Several *spo* mutants that synthesized but failed to incorporate DPA have been identified (221). Most of these were stage V mutants. Several of these, *spoVD*, *spoVE*, and possibly *spoVB*, probably have an indirect effect on DPA incorporation through their specific roles in cortex synthesis (see below). The remaining two, *spoVA* and *spoVK*, could, in principle, have a role in DPA or Ca^{2+} transport or both.

spoVA. The *spoVA* locus consists of a polycistronic operon, comprising at least five genes (86). It is expressed in the prespore beginning 2 to 3 h after the onset of sporulation, and it belongs to the σ^G regulon (Tables 1 and 2). Mutations in at least four of the *spoVA* genes abolish spore heat resistance, but partial resistance to lysozyme and organic solvents remain, in accordance with the presence of an intact spore coat (75, 86 [see below]). The properties of the predicted SpoVA proteins are compatible with the notion that they form a membrane-associated complex (86); some have stretches of amino acids that are rich in hydrophobic residues or rich in basic residues. Clearly, detailed biochemical analysis would be required to prove whether the SpoVA proteins participate in Ca^{2+}-DPA accumulation.

***spoVK* (*spoVJ*).** The *spoVK* locus was originally defined by transposon Tn*917* insertion mapping at 168° on the standard genetic map (240). Recently, it was discovered that the extensively characterized DNA cloned on the basis of complementation of a mutation called *spoVJ517* (119) actually corresponded to the *spoVK* locus (81), the *spoVJ* mutation having been incorrectly mapped to 250° (119). The phenotypic properties of *spoVK* mutants are very similar to those of *spoVA* mutants (75, 81), again suggesting a possible role in DPA transport. However, in contrast to *spoVA*, *spoVK* expression occurs in the mother cell (Table 1). Interestingly, this gene is transcribed from dual promoters recognized respectively by σ^E and σ^K (89). As with the *cotE* gene (323), this could serve to maintain the level of *spoVK* expression during the early and late phases of mother cell development. The *spoVK* locus encodes a single protein that contains an ATP-binding motif; conceivably the protein could use ATP hydrolysis to drive the uptake of Ca^{2+} or the transport of DPA into the prespore.

CORTEX SYNTHESIS

The spore cortex is one of the two most obvious morphological structures of the mature spore. It has been suggested that the cortex is responsible for either the establishment or the maintenance of the dehydrated state of the prespore, which contributes to some of the resistance properties of the mature spore, particularly to heat (reviewed in references 100 and 104). The cortex consists of PG whose structure is only slightly different from that of the PG in the vegetative cell wall. Cortex synthesis is likely to be, in the main, a function of the mother cell because it occurs on the inner surface of the outer prespore membrane. This is topologically equivalent to the site of normal cell wall synthesis on the outside of the cytoplasmic membrane of vegetative cells. Presumably, the precursors of cortex synthesis are the same as those used in vegetative PG synthesis. However, sporulation-specific proteins are likely to be involved in producing the modified PG of the cortex. Several genes belonging to the σ^E and σ^K regulons have been implicated in cortex synthesis because mutations in them produce fairly specific defects in cortex synthesis. Other genes are beginning to be identified on the basis that they encode proteins known to be involved in the final steps of PG synthesis, in particular the PBPs.

spo Genes Involved in Cortex Synthesis

***spoVD* and *spoVE*.** Two *spo* genes implicated in cortex synthesis on the basis of their mutant phenotypes (221), *spoVD* and *spoVE*, lie in a region of the chromosome that contains a cluster of genes involved in cell wall synthesis and cell division. The order of genes in *B. subtilis* is very similar to a cluster of genes with similar functions in *E. coli* (Fig. 8). The *spoVD* and *spoVE* products are closely related in amino acid sequence (>30% identity) to the products of the *E. coli pbpB* and *ftsW* genes, respectively, and their genes occupy similar positions in the gene cluster (48, 126, 149). The *spoVD* gene is regulated independently of the other genes in the cluster and is both expressed and required only during sporulation (48). Its product is homologous to that of the *pbpB* (*ftsI*) gene of *E. coli*, which is required specifically for cross-wall synthesis (135). However, closely related proteins, such as that encoded by the *pbpA* gene (PBP 2), are involved in cylindrical wall synthesis (i.e., extension in the long axis of the cell [for a review, see reference 194]). This fact tends to support the idea that *spoVD* could be involved in the synthesis of a sporulation-specific modified peptidoglycan.

The regulation of *spoVE* is unusual in that the gene is embedded in an operon that is essential for vegetative growth (49, 118). An additional σ^E-dependent promoter lies immediately upstream of *spoVE* and presumably allows this gene to be expressed during sporulation (117, 118). The *spoVE* and *ftsW* products are very similar to the *rodA* product (126, 149); mutations in these genes generate a round phenotype, suggesting a role in the synthesis of the cylindrical part of the cell wall (194). The *ftsW* mutations seem to affect cross-wall synthesis (194). However, despite the similar location of *spoVE* to *ftsW*, the *spoVE* product does not seem to be needed for vegetative growth or division (118). In fact, a stem-loop structure near the translation initiation site of *spoVE* could act to prevent the translation of *spoVE* mRNA initiated at the upstream promoter that is active during vegetative growth.

***spoVB* (*spoIIIF*).** Mutations in the *spoVB* gene give a phenotype in which coat formation is well advanced but the cortex is incomplete (124, 221, 224). The closely linked *spoIIIF* locus was defined on the basis of the *spoIIIF590* mutation (169). However, the strain carrying this mutation is now known to be a double mutant, whose major lesion lies in *spoVB* and confers the same phenotype as the classic *spoVB91* mutation (224). *spoVB* has been sequenced; it encodes a probable integral membrane protein of 518 amino acids (224), but it does not exhibit an obvious homology to genes known to be involved in cell wall metabolism. The *spoVB* promoter has not been fully characterized, but it also appears to belong to the σ^E regulon.

spoVG. Mutations in the *spoVG* gene, which begins to be expressed from the onset of sporulation (325), have a variety of phenotypic effects, some suggestive of a role in PG metabolism. Originally, deletion of the gene was reported to cause a minor defect in cortex synthesis (234). More recently, it has become apparent that *spoVG* mutations also act synergistically with *spoIIB* mutations to prevent the early steps of prespore engulfment (184; see above). Again, this may be due to an effect on PG turnover, but the precise biochemical defect caused by *spoVG* (or *spoIIB*) mutations is not yet known.

***gerJ* and *gerM*.** Two genes originally identified by defects in the intermediate stages of germination are now known also to be involved in sporulation. *gerJ* mutants are defective

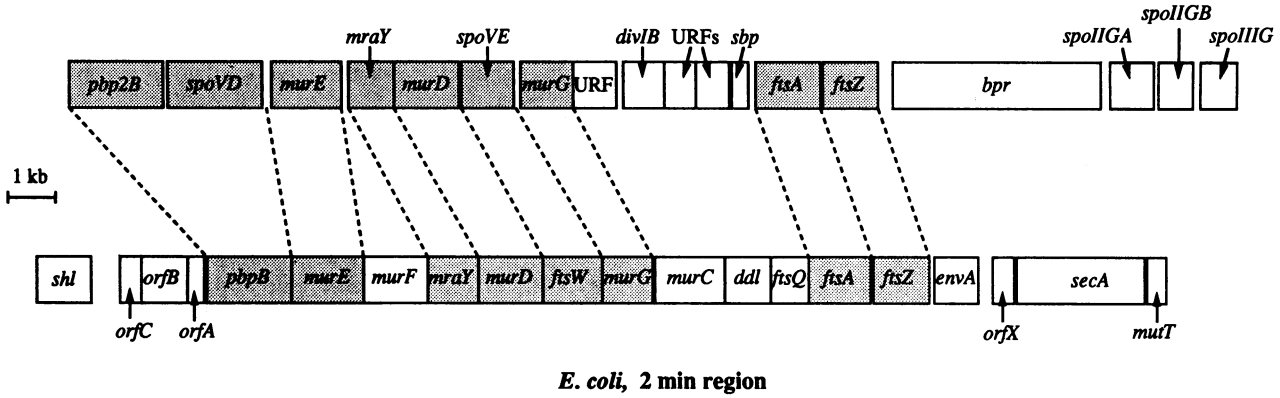

FIG. 8. Comparison of the *E. coli* and *B. subtilis* chromosomes in regions containing conserved clusters of cell wall synthesis and cell division genes. The information shown is derived in each case from more than 20 kb of contiguous DNA sequence. The DNA sequence of this region of the *E. coli* chromosome has recently been compiled by Yura et al. (320). The *B. subtilis* DNA sequences were from references 11, 12, 21, 25, 48, 49, 113, 118, 126, 151, 161, 191, 192, 243, 260, and 274. Genes with obvious homologs in the other organism are shaded, and broken lines are used to indicate the alignments. The sporulation-specific *spoVD* gene could have been derived by gene duplication and divergence because both it and the *pbp2B* gene immediately upstream are closely related to the *pbpB* gene of *E. coli* (21, 48). The *spoVE* gene, however, occupies the position of the *ftsW* gene of *E. coli*, which is essential for cell division (126, 149). Two of the *E. coli* cell division genes, *ftsQ* and *ddl*, do not seem to have homologs at the appropriate position in the *B. subtilis* chromosome. However, in the equivalent place lies at least one division gene, *divIB*, for which an *E. coli* homolog has not yet been found (12, 113).

in cortex synthesis, although they produce normal spore coats and accumulate DPA (302). The gene is expressed after about 90 min. The defect in cortex synthesis and the time of expression suggest that this gene could also belong to the σ^E regulon. *gerM* mutants are oligosporogenous. Some cells are blocked at stage II with defective septa; the cells that traverse stage II produce spores with minor defects in cortex synthesis and heat resistance, as well as defective germination (238). The timing of *gerM* synthesis ($t_{1.5}$) and its role in cortex synthesis again suggest that this gene may belong to the σ^E regulon. Indeed, upstream of the translational start of the gene are sequences closely resembling those of most σ^E-dependent genes (261). The predicted product of *gerM* would be a protein of about 21 kDa with no homology to known proteins. Interestingly, near the N terminus is a probable lipoprotein signal sequence, suggesting a location for the protein between the spore membranes and anchored to the outer membrane (261).

Penicillin-Binding Proteins

Binding of labelled penicillin has been used to identify a collection of membrane proteins that participate in cell wall synthesis in a wide range of bacteria (reviewed in reference 306). The PBPs can be divided into two major groups on the basis of size. The low-molecular-mass PBPs (~40 to 50 kDa) exhibit transpeptidase activity in vitro. The high-molecular-mass PBPs (~60 to 140 kDa) have this activity and, in addition, transglycosylase activity. Vegetative cells of *B. subtilis* have a collection of PBPs, which superficially resemble those of *E. coli*. Comparison of the PBPs of vegetative and sporulating cells has led to the identification of several proteins that are sporulation specific or that increase in abundance during sporulation (263, 290).

PBP 2B and PBP 3. At least two high-molecular-mass PBPs, PBP 2B and PBP 3, increase in abundance during sporulation (263). The gene for PBP 2B lies immediately upstream of *spoVD* (21, 48) and could thus correspond to the functional homolog of the *pbpB* gene of *E. coli*. If so, one might expect it to be required for septum formation early in sporulation. Thus, its possible role in cortex synthesis has yet to be established. The gene encoding PBP 3 has not yet been isolated to my knowledge.

dacB. A major low-molecular-mass PBP, called PBP 5a (290) or PBP 5* (263), which is the major sporulation-specific PBP, has been identified by two groups. As expected for a low-molecular-mass PBP, this protein exhibits D-alanine carboxypeptidase activity in vitro (289). The gene encoding this protein, *dacB*, is expressed specifically during sporulation, exhibits a dependence pattern suggestive of transcription by $E\sigma^E$, and has possible −10 and −35 promoter sequences of the appropriate type (23). The protein product is specifically associated with the developing spore (290), in particular with the outer prespore membrane (24). Gene disruption experiments indicate that this protein is necessary for the formation of heat-resistant spores, in accordance with a defect in cortex synthesis (22).

dacA. A closely related protein, PBP 5, is present in vegetative cells and in the outer membrane of the forespore (24, 290). Part of the gene encoding this protein, *dacA*, has been cloned (291). Although one group has reported that disruption of the *dacA* gene led to an altered sporulation morphology and reduced heat resistance (291), more recent results indicate that this gene is not required for sporulation or vegetative growth (22).

Primordial Germ Cell Wall Synthesis

There is some evidence for a thin inner layer of cortex, of slightly different composition, which is sometimes called the primordial germ cell wall (PCW) (287). The existence of such a distinct layer is well established in the closely related organism *B. sphaericus*, for which it has been demonstrated that the true cortex and the PCW are synthesized by

TABLE 3. *cot* genes and their products and regulation

Gene	Mol mass (kDa)[a]	Map position	Effect of mutation on:		Location of protein[b]	Comments	Reference(s)
			Lysozyme resistance	Germination[c]			
cotA	65	52	+	+	O	Also called *pig*; produces brown pigment of spores	56, 239
cotB	59	290	+	+	O	Closely related protein of 34 kDa	56
cotC	12	168	+	+	O	Rich in tyrosine and lysine; closely related protein of 18 kDa	56
cotD	11	200	+	(+)	I	Contains several cysteine and methionine residues	56
cotE	24 (21)	145	−	(+)	I	Absence of CotE blocks incorporation of various other coat proteins into the coat; outer coat absent but inner lamellar coat more or less normal; abundant highly cross-linked protein, possibly with peroxidase activity	54, 321
cotF	5, 8 (19)	349	+	+	?	Protein soluble in alkali; 5- and 8-kDa products generated by proteolytic cleavage of larger precursor	42
cotT	7.8 (12)	108	+	(+)	I	Only seven different amino acids present; rich in glycine, proline, and tyrosine; processed from larger precursor	8, 17, 18

[a] Observed molecular mass of protein. Molecular mass calculated from DNA sequence is shown in parentheses.
[b] O, outer layer; I, inner layer.
[c] (+), slightly impaired germination (*cotE*); slow germination (*cotD*); impaired germination with AGFK but not alanine (*cotT*).

enzymes acting specifically in the mother cell and prespore, respectively (288). The PCW is thought to act as the precursor to the new cell wall following germination and outgrowth, whereas the cortex is degraded on germination. As yet, no prespore genes involved in PCW synthesis have been defined by *spo* mutations, perhaps because they are also involved in vegetative PG synthesis. A gene apparently encoding a third putative low-molecular-mass PBP, designated *dacF*, was recently found immediately upstream of the *spoIIA* operon (310). Disruption of this gene had no effect on sporulation, so if its product is involved in PCW synthesis, its function must be redundant.

Cortex synthesis is evidently a complicated process involving several genes. Some of the genes seem to have been derived by duplication of genes known to be involved in vegetative cell wall synthesis. Still others appear to be bifunctional and involved in both growth and sporulation; presumably these will turn out to have complex regulation with multiple promoters. A major problem in interpreting these findings is that the in vivo functions of the various classes of PBP are still unknown. However, study of the sporulation-specific enzymes and the modified forms of PG they produce should improve our general knowledge of this interesting and important group of proteins.

SYNTHESIS AND ASSEMBLY OF SPORE COAT PROTEINS

The spore coat represents one of the most conspicuous of the morphological structures formed during sporulation. It consists of at least a dozen different proteins deposited in concentric layers on the outer face of the outer prespore membrane. Pulse-labeling and surface iodination have been used to study the timing of spore coat protein synthesis and the order of assembly of the proteins (144). These experiments showed that the proteins were deposited in a defined order but that this order did not correspond closely to the order of their synthesis. Moreover, the assembly of the spore coat has been shown to occur largely in the absence of protein synthesis, indicating that self-assembly processes play an important part in the formation of the coat (142). The spore coat thus provides an interesting and experimentally tractable model for studies of the synthesis and assembly of a complex morphological structure.

Spore Coat Protein Genes

Seven spore coat protein genes have been cloned and characterized in detail so far. The properties of these genes and their products are summarized in Table 3. The genes that have been studied so far are mainly those encoding the most abundant proteins of the spore coat, which were the easiest to purify, and which lie mainly in the outer layers of the coat. Some were isolated on the basis of their unusual solubility at alkaline pH. The *cotA* gene was also identified as the site of mutations preventing the synthesis of the characteristic brown pigment associated with the late stages of sporulation (56). The absence of this pigment was used as the basis for identification of many of the *spo* mutants (179).

Regulation of Spore Coat Protein Synthesis

Pulse-labeling studies have shown that spore coat proteins differ greatly in their times of synthesis: although some proteins begin to be synthesized about 2 h after the initiation of sporulation, others are not labeled until t_5 or so (144). With our knowledge of the various changes that occur in the transcriptional apparatus during sporulation, the different times of spore coat protein synthesis can now be partially explained (323). Those expressed earlier in sporulation are likely to have σ^E-dependent promoters, and their precise timing could be modulated by the action of SpoIIID (see above). The *cotE* gene falls into this category, but it has an additional σ^K-dependent promoter that presumably allows its synthesis to continue when σ^K appears. The other well-characterized *cot* genes all have σ^K-dependent promoters, but they show considerable variation in time of expression (Table 1). The SpoIIID protein may be responsible for some of these effects, in addition to its role in the regulation of some σ^E-dependent genes. It can repress transcription from

the *cotD* promoter in vitro. The disappearance of SpoIIID during the later stages of sporulation (cited in reference 322) may thus be important in the release of transcription of certain spore coat protein genes.

Role of *gerE*. The *gerE* gene encodes a small DNA-binding protein that seems to exert control over the genes of the σ^K regulon in a manner similar to the role of the SpoIIID protein in controlling the σ^E regulon. The main difference is that *gerE* does not appear to autoregulate (41). On the basis of the effects of a *gerE* mutation, the GerE protein negatively regulates the *cotA* and possibly *cotT* genes (8, 239), and positively regulates the *cotB*, *cotC*, and *cotD* genes (323) (Tables 1 and 2). Thus, like SpoIIID, GerE appears to be capable of both positive and negative regulation of transcription, and this has recently been confirmed by in vitro experiments (322). The GerE protein thus appears to fine-tune the timing and perhaps the rate of expression of genes determining spore coat synthesis.

Proteolysis. Earlier work on spore coat synthesis had suggested that proteases might play an important role in the assembly of the spore coat (139, 143, 241). So far, two of the characterized spore coat genes, *cotF* and *cotT*, seem to encode precursor proteins that need to be processed (8, 42). As yet, neither the relevant proteases nor their genes have been characterized in detail.

Spore Coat Assembly

The major insights into spore coat assembly have been derived from studies of mutants (Fig. 1). Perhaps surprisingly, mutations in most of the *cot* genes have little or no effect on sporulation or germination: the spore coat apparently assembles normally, showing only the absence of the single protein. On the other hand, *cotE* mutant spores show normal refractility, but they are lysozyme sensitive and have minor changes in germination properties (321). Preparations of spore coat proteins are deficient not only in the CotE product but also in several other major proteins synthesized and deposited during the late stages of sporulation (e.g., the proteins encoded by the *cotA*, *cotB*, and *cotC* genes). Electron microscopy shows that *cotE* mutant spores possess the inner lamellar layer of wild-type spore coats but not the outer electron-dense layer. The absence of the outer coat layers in this mutant accords with the previous suggestion that resistance to lysozyme is a function of this layer (140, 142). The absence of proteins other than CotE is not due to an effect at the level of gene expression, so it must result from a failure in assembly (321). This raises the possibility that the CotE protein is deposited on the outside surface of the inner coat, where it serves as a basement protein on which the proteins of the outer coat assemble (321). Recent results, however, indicate that the CotE protein is abundant and is deposited in the coat in a highly cross-linked form (175). Amino acid sequence similarity suggests that the CotE protein may have a peroxidase activity. This cross-linking activity could play an important role in the covalent assembly of the outer spore coat (54).

Two well-characterized proteins encoded by the *cotD* and *cotT* genes are probably assembled into the inner spore coat, since they are present in the coats of *cotE* mutant spores (17). This conclusion is supported by the observations that *cotT* mutant spores are visibly deficient in the striated inner coat and that overexpression of the *cotT* gene leads to an apparent increase in the thickness of the inner coat (17). Interestingly, overproduction of the CotD or CotT proteins, or a mutation inactivating the *cotT* gene, has mild effects on germination properties (17, 18), suggesting that the inner coat is important in the permeability or response to germinants.

Mutations in the *spoIVA* gene have perhaps the most dramatic effect on spore coat assembly. Much spore coat material is made, but the proteins seem to assemble in cytoplasmic masses rather than being deposited on the surface of the prespore (36, 232). The amino acid sequence of *spoIVA* indicates an extremely acidic protein, and it contains a motif suggestive of ATP binding (267). However, SpoIVA is also required for cortex formation, which suggests that the assembly of the spore coat may be coupled somehow to synthesis of the cortex. In this case the SpoIVA protein would have no direct role in spore coat formation and its absence would affect coat assembly indirectly.

There is still much to be learned about the synthesis and assembly of the spore coat, but considerable progress has been made. Several of the major coat protein genes have been cloned, and their temporal regulation is understood in some detail. The phenotypic effects of engineered mutants have revealed aspects of the assembly process and of the different functions of the two major layers of the coat.

CONCLUSIONS

Although *B. subtilis* sporulation involves only two differentiated cells, its regulatory mechanisms are complex and sophisticated. Many parallels between sporulation and developmental processes of other organisms are apparent. It is therefore useful to consider the extent to which we now understand the molecular basis of this developmental system and the prospects for future progress.

We certainly know a great deal about the developmental genes of *B. subtilis*, mainly because this organism is outstandingly amenable to molecular genetic analysis. We know, at least in outline, the program of expression of many of the genes that regulate sporulation and understand the mechanisms responsible for many of the genetic interactions in some detail. Most of the genes have been sequenced, so their products are known, at least at the primary sequence level. It should not be long before the remaining genes have been characterized in this way.

Probably the most interesting question posed by sporulation concerns the establishment of differential gene expression after septation. The way in which two cells with identical genomes initiate different programs of gene expression is crucial in developmental biology. A partial understanding of this problem in *B. subtilis* is already evident. It seems to involve the regulated distribution of two key transcription factor activities, σ^E and σ^F, to different cells after septation. Indeed, sigma factors seem to control several of the key steps in sporulation. In this respect they appear to be functionally equivalent to the "master" regulator genes, such as MyoD, of eukaryotes (285). Each sigma factor can be considered to determine a specific cell type at any given time in development.

Perhaps not surprisingly, the regulation of sigma factor activity is complex. All of the sigma factors are subject to multiple levels of control, and in each case at least one level of regulation could involve feedback from events occurring at the morphological level. Such checkpoints (114) would ensure that gene expression remained in register with morphological development. However, to date, we have little detailed information on the mechanisms involved. In fact, most of these regulatory mechanisms could also be viewed as intercompartmental signals that keep the genetic pro-

grams of the differentiating sister cells in register, almost irrespective of morphological events. Thus, σ^E processing in the mother cell seems to be triggered by the action of σ^F activity in the prespore; transcription of *spoIIIG* and activation of σ^G in the prespore are dependent on gene expression in the mother cell; and σ^K synthesis in the mother cell is triggered at least partly by gene expression in the prespore. Of course, these regulatory pathways could subserve both functions.

Other aspects of *B. subtilis* development are less well understood than the genetic program. Progress is beginning to be made in elucidating the way in which specific gene products bring about morphological change. This is particularly apparent in the case of the formation of the spore coat, which is very amenable to this kind of study. It is already clear that two classes of coat protein can be distinguished. One class (e.g., CotE) is required for the assembly of the coat: in the absence of this protein, other proteins fail to assemble into the coat correctly. The second class has no such essential structural role: the absence of an individual protein of this class has no overall effect on the assembly of the remaining proteins, but the spore may be defective in a specific germination response or resistance property (CotD is an example).

Other structural changes may be less easy to characterize but are worthy of more attention. Cortex formation is a modified form of cell wall synthesis, and its study may aid understanding this poorly understood phenomenon in vegetative cells. Similarly, progress in understanding the formation of the asymmetric spore septum should shed light on the important problem of bacterial cell division. For example, comparative studies of the precise positioning of the vegetative septum in the midpoint of the cell and the asymmetric positioning during sporulation should prove valuable. The unique phenomenon of engulfment may prove less tractable, but its study is eminently worthwhile, not least because it has certain features in common with endocytosis in eukaryotes. Again, genes involved in engulfment are beginning to be identified.

Now that many of the sporulation genes have been cloned and characterized, it is also possible to begin to consider the evolution of sporulation. Many of the genes involved appear to have arisen by gene duplication and divergence from genes that must have been present in the nonsporulating progenitor (48, 66, 70, 127). There are now numerous examples of sporulation genes that probably originated in this way. Other genes needed for both vegetative growth and sporulation have probably been recruited for the latter purpose by the development of complex promoters that can be recognized by more than one form of RNA polymerase (29, 102, 103, 118). These systems are reminiscent of the complex promoters of higher organisms which allow genes to be expressed in different tissues or at different stages of development.

It is clear that a great deal of progress has been made in elucidating the molecular basis for this simple developmental system but that there is still much scope for further work. The powerful genetic and biochemical approaches that can be applied to *B. subtilis* should ensure that solutions to some of the most interesting problems posed by this developmental system should soon be forthcoming, and this is likely to have important implications for other developmental systems.

ACKNOWLEDGMENTS

I am indebted to Joel Mandelstam and Michael Yudkin for invaluable discussions and constructive criticisms of the manuscript. Many other people made helpful comments and suggestions and communicated unpublished results, for which I am grateful: particularly, R. Allmansberger, M. J. Bramucci, C. E. Buchanan, B. Corfe, S. M. Cutting, D. Foulger, J. A. Hoch, R. Losick, A. Moir, C. P. Moran, Jr., P. J. Piggot, P. Setlow, D. van Sinderen, R. G. Wake, and P. J. Youngman.

Work in this laboratory is supported by the Science and Engineering Research Council (grants GR/F/75261 and GR/G/47360), the Medical Research Council (grant G8823935CB), and the Agriculture and Food Research Council (AT43/543).

REFERENCES

1. **Adams, L. F., K. L. Brown, and H. R. Whiteley.** 1991. Molecular cloning and characterization of two genes encoding sigma factors that direct transcription from a *Bacillus thuringiensis* crystal protein gene promoter. J. Bacteriol. **173:**3846–3854.
2. **Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree.** 1967. Miniature *Escherichia coli* cells deficient in DNA. Proc. Natl. Acad. Sci. USA **57:**321–326.
3. **Akrigg, A.** 1978. Purification and properties of a manganese-stimulated deoxyribonuclease produced during sporulation of *Bacillus subtilis*. Biochem. J. **172:**69–76.
4. **Akrigg, A., and J. Mandelstam.** 1978. Extracellular manganese-stimulated deoxyribonuclease as a marker event in sporulation of *Bacillus subtilis*. Biochem. J. **172:**63–67.
5. **Andreoli, A. J., J. Saranto, P. A. Baecker, S. Suehiro, E. Escamilla, and A. Steiner.** 1975. Biochemical properties of forespores isolated from *Bacillus cereus*, p. 418–424. *In* P. Gerhardt, R. N. Costilow, and H. L. Sadoff (ed.), Spores VI. American Society for Microbiology, Washington, D.C.
6. **Andreoli, A. J., S. Suehiro, D. Sakiyama, J. Takemoto, E. Vivanco, J. C. Lara, and M. C. Klute.** 1973. Release and recovery of forespores from *Bacillus cereus*. J. Bacteriol. **115:**1159–1166.
7. **Antoniewski, C., B. Savelli, and P. Stragier.** 1990. The *spoIIJ* gene, which regulates early developmental steps in *Bacillus subtilis*, belongs to a class of environmentally responsive genes. J. Bacteriol. **172:**86–93.
8. **Aronson, A. I., H.-Y. Song, and N. Bourne.** 1989. Gene structure and precursor processing of a novel *Bacillus subtilis* spore coat protein. Mol. Microbiol. **3:**437–444.
9. **Bach, M. L., and C. Gilvarg.** 1966. Biosynthesis of dipicolinic acid in sporulating *Bacillus megaterium*. J. Biol. Chem. **241:**4563–4564.
10. **Balassa, G., P. Milhaud, E. Raulet, M. T. Silva, and J. C. F. Sousa.** 1979. A *Bacillus subtilis* mutant requiring dipicolinic acid for the development of heat-resistant spores. J. Gen. Microbiol. **110:**365–379.
11. **Beall, B., M. Lowe, and J. Lutkenhaus.** 1988. Cloning and characterization of *Bacillus subtilis* homologs of *Escherichia coli* cell division genes *ftsZ* and *ftsA*. J. Bacteriol. **170:**4855–4864.
12. **Beall, B., and J. Lutkenhaus.** 1989. Nucleotide sequence and insertional inactivation of a *Bacillus subtilis* gene that affects cell division, sporulation, and temperature sensitivity. J. Bacteriol. **171:**6821–6834.
13. **Beall, B., and J. Lutkenhaus.** 1991. FtsZ in *Bacillus subtilis* is required for vegetative septation and for asymmetric septation during sporulation. Genes Dev. **5:**447–455.
14. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12:**721–732.
15. **Bi, E., and J. Lutkenhaus.** 1990. FtsZ regulates frequency of cell division in *Escherichia coli*. J. Bacteriol. **172:**2765–2768.
16. **Bookstein, C., C. W. Edwards, N. V. Kapp, and F. M. Hulett.** 1990. The *Bacillus subtilis* 168 alkaline phosphatase III gene: impact of a *phoAIII* mutation on total alkaline phosphatase synthesis. J. Bacteriol. **172:**3730–3737.
17. **Bourne, N., P. C. Fitz-James, and A. I. Aronson.** 1991. Struc-

tural and germination defects of *Bacillus subtilis* spores with altered contents of a spore coat protein. J. Bacteriol. **173**:6618–6625.
18. **Bourne, N., T.-S. Huang, and A. I. Aronson.** 1990. Properties of *Bacillus subtilis* spores with alterations in spore coat structure, p. 329–339. *In* M. M. Zukowski, A. T. Ganesan, and J. A. Hoch (ed.), Genetics and biotechnology of bacilli, vol. 3. Academic Press, Inc., New York.
19. **Bouvier, J., P. Stragier, C. Bonamy, and J. Szulmajster.** 1984. Nucleotide sequence of the *spo0B* gene of *Bacillus subtilis* and regulation of its expression. Proc. Natl. Acad. Sci. USA **81**:7012–7016.
20. **Bramucci, M. G.** 1992. Personal communication.
21. **Buchanan, C. E., R. A. Daniel, and J. Errington.** 1992. Unpublished data.
22. **Buchanan, C. E., and A. Gustafson.** 1992. Mutagenesis and mapping of the gene for a sporulation-specific penicillin-binding protein in *Bacillus subtilis*. J. Bacteriol. **174**:5430–5435.
23. **Buchanan, C. E., and M.-L. Ling.** 1992. Isolation and sequence analysis of *dacB*, which encodes a sporulation-specific penicillin-binding protein in *Bacillus subtilis*. J. Bacteriol. **174**:1717–1725.
24. **Buchanan, C. E., and S. L. Neyman.** 1986. Correlation of penicillin-binding protein composition with different functions of two membranes in *Bacillus subtilis* forespores. J. Bacteriol. **165**:498–503.
25. **Bugaichuk, U. D., and P. J. Piggot.** 1986. Nucleotide sequence of the *Bacillus subtilis* developmental gene *spoVE*. J. Gen. Microbiol. **132**:1883–1890.
26. **Burbulys, D., K. A. Trach, and J. A. Hoch.** 1991. Initiation of sporulation in B. subtilis is controlled by a multicomponent phosphorelay. Cell **64**:545–552.
27. **Carlson, H. C., and W. G. Haldenwang.** 1989. The σ^E subunit of *Bacillus subtilis* RNA polymerase is present in both forespore and mother cell compartments. J. Bacteriol. **171**:2216–2218.
28. **Carter, H. L., III, and C. P. Moran, Jr.** 1986. New RNA polymerase σ factor under *spo0* control in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **83**:9438–9442.
29. **Carter, H. L., III, L.-F. Wang, R. H. Doi, and C. P. Moran, Jr.** 1988. *rpoD* operon promoter used by σ^H-RNA polymerase in *Bacillus subtilis*. J. Bacteriol. **170**:1617–1621.
30. **Chesnut, R. S., C. Bookstein, and F. M. Hulett.** 1991. Separate promoters direct expression of *phoAIII*, a member of the *Bacillus subtilis* alkaline phosphatase multigene family, during phosphate starvation and sporulation. Mol. Microbiol. **5**:2181–2190.
31. **Chibazakura, T., F. Kawamura, and H. Takahashi.** 1991. Differential regulation of *spo0A* transcription in *Bacillus subtilis*: glucose represses promoter switching at the initiation of sporulation. J. Bacteriol. **173**:2625–2632.
32. **Clarke, S., I. Lopez-Diaz, and J. Mandelstam.** 1986. Use of *lacZ* gene fusions to determine the dependence pattern of the sporulation gene *spoIID* in *spo* mutants of *Bacillus subtilis*. J. Gen. Microbiol. **132**:2987–2994.
33. **Clarke, S., and J. Mandelstam.** 1980. Dissociation of an early event in sporulation from chromosome replication in *Bacillus subtilis*. J. Gen. Microbiol. **121**:487–490.
34. **Clarke, S., and J. Mandelstam.** 1987. Regulation of stage II of sporulation in *Bacillus subtilis*. J. Gen. Microbiol. **133**:2371–2380.
35. **Connors, M. J., S. Howard, J. Hoch, and P. Setlow.** 1986. Determination of the chromosomal locations of four *Bacillus subtilis* genes which code for a family of small, acid-soluble spore proteins. J. Bacteriol. **166**:412–416.
36. **Coote, J. G.** 1972. Sporulation in *Bacillus subtilis*. Characterization of oligosporogenous mutants and comparison of their phenotypes with those of asporogenous mutants. J. Gen. Microbiol. **71**:1–15.
37. **Coppolecchia, R., H. DeGrazia, and C. P. Moran, Jr.** 1991. Deletion of *spoIIAB* blocks endospore formation in *Bacillus subtilis* at an early stage. J. Bacteriol. **173**:6678–6685.
38. **Corfe, B., R. L. Sammons, A. Moir, and D. A. Smith.** 1992. Unpublished data.
39. **Cutting, S., and J. Mandelstam.** 1986. The nucleotide sequence and the transcription during sporulation of the *gerE* gene of *Bacillus subtilis*. J. Gen. Microbiol. **132**:3013–3024.
40. **Cutting, S., V. Oke, A. Driks, R. Losick, S. Lu, and L. Kroos.** 1990. A forespore checkpoint for mother cell gene expression during development in B. subtilis. Cell **62**:239–250.
41. **Cutting, S., S. Panzer, and R. Losick.** 1989. Regulatory studies on the promoter for a gene governing synthesis and assembly of the spore coat in *Bacillus subtilis*. J. Mol. Biol. **207**:393–404.
42. **Cutting, S., L. Zheng, and R. Losick.** 1991. Gene encoding two alkali-soluble components of the spore coat from *Bacillus subtilis*. J. Bacteriol. **173**:2915–2919.
43. **Cutting, S. M., A. Driks, R. Schmidt, B. Kunkel, and R. Losick.** 1991. Forespore-specific transcription of a gene in the signal transduction pathway that governs Pro-σ^K processing in *Bacillus subtilis*. Genes Dev. **5**:456–466.
44. **Cutting, S. M., S. Roels, and R. Losick.** 1991. Sporulation operon *spoIVF* and the characterization of mutations that uncouple mother-cell from forespore gene expression in *Bacillus subtilis*. J. Mol. Biol. **221**:1237–1256.
45. **Cutting, S. M., and P. B. Vander Horn.** 1990. Genetic analysis, p. 27–74. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley and Sons Ltd., Chichester, United Kingdom.
46. **Dancer, B. N.** 1981. Control of sporulation in fused protoplasts of *Bacillus subtilis* 168. J. Gen. Microbiol. **126**:29–36.
47. **Dancer, B. N., and J. Mandelstam.** 1981. Complementation of sporulation mutations in fused protoplasts of *Bacillus subtilis*. J. Gen. Microbiol. **123**:17–26.
48. **Daniel, R. A., S. E. Drake, C. E. Buchanan, D. Foulger, R. Scholle, and J. Errington.** Unpublished data.
49. **Daniel, R. A., and J. Errington.** 1993. DNA sequence of the *murE-murD* region of *Bacillus subtilis* 168. J. Gen. Microbiol. **139**:361–370.
50. **Daniel, R. A., and J. Errington.** Submitted for publication.
51. **Dawes, I. W., D. Kay, and J. Mandelstam.** 1971. Determining effect of growth medium on the shape and position of daughter chromosomes and on sporulation in *Bacillus subtilis*. Nature (London) **230**:567–569.
52. **Dawes, I. W., and J. Mandelstam.** 1970. Sporulation of *Bacillus subtilis* in continuous culture. J. Bacteriol. **103**:529–535.
53. **De Lencastre, H., and P. J. Piggot.** 1979. Identification of different sites of expression for *spo* loci by transformation of *Bacillus subtilis*. J. Gen. Microbiol. **114**:377–389.
54. **Diets, T.** 1992. Personal communication.
55. **Dion, P., and J. Mandelstam.** 1980. Germination properties as marker events characterizing later stages of *Bacillus subtilis* spore formation. J. Bacteriol. **141**:786–792.
56. **Donovan, W., L. Zheng, K. Sandman, and R. Losick.** 1987. Genes encoding spore coat polypeptides from *Bacillus subtilis*. J. Mol. Biol. **196**:1–10.
57. **Driks, A., and R. Losick.** 1991. Compartmentalized expression of a gene under the control of sporulation transcription factor σ^E in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **88**:9934–9938.
58. **Dubnau, D.** 1991. Genetic competence in *Bacillus subtilis*. Microbiol. Rev. **55**:395–424.
59. **Dubnau, E., J. Weir, G. Nair, L. Carter III, C. P. Moran, Jr., and I. Smith.** 1988. *Bacillus* sporulation gene *spo0H* codes for σ^{30} (σ^H). J. Bacteriol. **170**:1054–1062.
60. **Dunn, G., P. Jeffs, N. H. Mann, D. M. Torgersen, and M. Young.** 1978. The relationship between DNA replication and the induction of sporulation in *Bacillus subtilis*. J. Gen. Microbiol. **108**:189–195.
61. **Dunn, G., D. M. Torgersen, and J. Mandelstam.** 1976. Order of expression of genes affecting septum location during sporulation of *Bacillus subtilis*. J. Bacteriol. **125**:776–779.
62. **Ellar, D. J.** 1978. Spore specific structures and their function, p. 295–325. *In* R. Y. Stanier, H. J. Rogers, and J. B. Ward (ed.), Relations between structure and function in the prokaryotic cell. Cambridge University Press, Cambridge.
63. **Ellar, D. J., and J. A. Postgate.** 1974. Characterization of

forespores isolated from *Bacillus megaterium* at different stages of development into mature spores, p. 21–40. *In* A. N. Barker, G. W. Gould, and J. Wolf (ed.), Spore research 1973. Academic Press Ltd., London.

64. **Errington, J.** 1990. Regulation of gene expression during sporulation in *Bacillus subtilis*, p. 667–677. *In* H. Heslot, J. Davies, J. Florent, L. Bobichon, G. Durand, and L. Penasse (ed.), Genetics of industrial microorganisms. Proceedings of the Sixth International Symposium, vol. II. Société Française de Microbiologie, Paris.
65. **Errington, J.** 1990. Gene cloning techniques, p. 175–220. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley and Sons Ltd., Chichester, United Kingdom.
66. **Errington, J.** 1991. Possible intermediate steps in the evolution of a prokaryotic developmental system. Proc. R. Soc. London Ser. B **244:**117–121.
67. **Errington, J.** 1992. Unpublished data.
68. **Errington, J., L. Appleby, R. A. Daniel, H. Goodfellow, S. R. Partridge, and M. D. Yudkin.** 1992. Structure and function of the *spoIIIJ* gene of *Bacillus subtilis*: a vegetatively expressed gene that is essential for σ^G activity at an intermediate stage of sporulation. J. Gen. Microbiol. **138:**2609–2618.
69. **Errington, J., S. M. Cutting, and J. Mandelstam.** 1988. Branched pattern of regulatory interactions between late sporulation genes in *Bacillus subtilis*. J. Bacteriol. **170:**796–801.
70. **Errington, J., P. Fort, and J. Mandelstam.** 1985. Duplicated sporulation genes in bacteria: implications for simple developmental systems. FEBS Lett. **188:**184–188.
71. **Errington, J., D. Foulger, N. Illing, S. R. Partridge, and C. M. Stevens.** 1990. Regulation of differential gene expression during sporulation in *Bacillus subtilis*, p. 257–267. *In* M. Zukowski, A. T. Ganesan, and J. A. Hoch (ed.), Genetics and biotechnology of bacilli, vol. III. Academic Press, Inc., San Diego, Calif.
72. **Errington, J., and N. Illing.** 1992. Establishment of cell-specific transcription during sporulation in *Bacillus subtilis*. Mol. Microbiol. **6:**689–695.
73. **Errington, J., and D. Jones.** 1987. Cloning in *Bacillus subtilis* by transfection with bacteriophage vector ϕ105J27: isolation and preliminary characterization of transducing phages for 23 sporulation loci. J. Gen. Microbiol. **133:**493–502.
74. **Errington, J., and J. Mandelstam.** 1983. Variety of sporulation phenotypes resulting from mutations in a single regulatory locus, *spoIIA*, in *Bacillus subtilis*. J. Gen. Microbiol. **129:**2091–2101.
75. **Errington, J., and J. Mandelstam.** 1984. Genetic and phenotypic characterization of a cluster of mutations in the *spoVA* locus of *Bacillus subtilis*. J. Gen. Microbiol. **130:**2115–2121.
76. **Errington, J., and J. Mandelstam.** 1986. Use of a *lacZ* gene fusion to determine the dependence pattern and the spore compartment expression of sporulation operon *spoVA* in *spo* mutants of *Bacillus subtilis*. J. Gen. Microbiol. **132:**2977–2985.
77. **Errington, J., and J. Mandelstam.** 1986. Use of a *lacZ* gene fusion to determine the dependence pattern of sporulation operon *spoIIA* in *spo* mutants of *Bacillus subtilis*. J. Gen. Microbiol. **132:**2967–2976.
78. **Errington, J., R. Scholle, S. Goo, and M. D. Yudkin.** 1992. Unpublished data.
79. **Errington, J., and R. G. Wake.** 1991. Chromosome strand segregation during sporulation in *Bacillus subtilis*. Mol. Microbiol. **5:**1145–1149.
80. **Errington, J., L. Wootten, J. C. Dunkerley, and D. Foulger.** 1989. Differential gene expression during sporulation in *Bacillus subtilis*: regulation of the *spoVJ* gene. Mol. Microbiol. **3:**1053–1060.
81. **Fan, N., S. M. Cutting, and R. Losick.** 1992. Characterization of the *Bacillus subtilis* sporulation gene *spoVK*. J. Bacteriol. **174:**1053–1054.
82. **Feavers, I. M., J. Foulkes, B. Setlow, D. Sun, W. Nicholson, P. Setlow, and A. Moir.** 1990. The regulation of transcription of the *gerA* spore germination operon of *Bacillus subtilis*. Mol. Microbiol. **4:**275–282.
83. **Felix, J., and D. G. Lundgren.** 1972. Some membrane characteristics of *Bacillus cereus* during growth and sporulation, p. 35–44. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores V. American Society for Microbiology, Washington, D.C.
84. **Ferrari, F. A., K. Trach, and J. A. Hoch.** 1985. Sequence analysis of the *spo0B* locus reveals a polycistronic transcription unit. J. Bacteriol. **161:**556–562.
85. **Ferrari, F. A., K. Trach, D. Le Coq, J. Spence, E. Ferrari, and J. A. Hoch.** 1985. Characterization of the *spo0A* locus and its deduced product. Proc. Natl. Acad. Sci. USA **82:**2647–2651.
86. **Fort, P., and J. Errington.** 1985. Nucleotide sequence and complementation analysis of a polycistronic sporulation operon, *spoVA*, in *Bacillus subtilis*. J. Gen. Microbiol. **131:**1091–1105.
87. **Fort, P., and P. J. Piggot.** 1984. Nucleotide sequence of sporulation locus *spoIIA* in *Bacillus subtilis*. J. Gen. Microbiol. **130:**2147–2153.
88. **Foulger, D., and J. Errington.** 1989. The role of the sporulation gene *spoIIIE* in the regulation of prespore-specific gene expression in *Bacillus subtilis*. Mol. Microbiol. **3:**1247–1255.
89. **Foulger, D., and J. Errington.** 1991. Sequential activation of dual promoters by different sigma factors maintains *spoVJ* expression during successive developmental stages of *Bacillus subtilis*. Mol. Microbiol. **5:**1363–1373.
90. **Foulger, D., and J. Errington.** 1992. Unpublished data.
91. **Francesconi, S. C., T. J. MacAlister, B. Setlow, and P. Setlow.** 1988. Immunoelectron microscopic localization of small, acid-soluble spore proteins in sporulating cells of *Bacillus subtilis*. J. Bacteriol. **170:**5963–5967.
92. **Freese, E.** 1981. Initiation of bacterial sporulation, p. 1–12. *In* H. S. Levinson, A. L. Sonenshein, and D. J. Tipper (ed.), Sporulation and germination. American Society for Microbiology, Washington, D.C.
93. **Freese, E., and J. Heinze.** 1984. Metabolic and genetic control of bacterial sporulation, p. 101–172. *In* A. Hurst and G. W. Gould (ed.), The bacterial spore, vol. 2. Academic Press Ltd., London.
94. **Fujita, Y., R. Ramaley, and E. Freese.** 1977. Location and properties of glucose dehydrogenase in sporulating cells and spores of *Bacillus subtilis*. J. Bacteriol. **132:**282–293.
95. **Fürbass, R., M. Gocht, P. Zuber, and M. A. Marahiel.** 1991. Interaction of AbrB, a transcriptional regulator from *Bacillus subtilis* with the promoters of the transition state-activated genes *tycA* and *spoVG*. Mol. Gen. Genet. **225:**347–354.
96. **Gardella, T., H. Moyle, and M. M. Susskind.** 1989. A mutant *Escherichia coli* σ^{70} subunit of RNA polymerase with altered promoter specificity. J. Mol. Biol. **206:**579–590.
97. **Gaur, N. K., K. Cabane, and I. Smith.** 1988. Structure and expression of the *Bacillus subtilis sin* operon. J. Bacteriol. **170:**1046–1053.
98. **Gaur, N. K., E. Dubnau, and I. Smith.** 1986. Characterization of a cloned *Bacillus subtilis* gene that inhibits sporulation in multiple copies. J. Bacteriol. **168:**860–869.
99. **Gaur, N. K., J. Oppenheim, and I. Smith.** 1991. The *Bacillus subtilis sin* gene, a regulator of alternate developmental processes, codes for a DNA-binding protein. J. Bacteriol. **173:**678–686.
100. **Gerhardt, P., and R. E. Marquis.** 1989. Spore thermoresistance mechanisms, p. 43–63. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of procaryotic development: a structural and functional analysis of sporulation and germination. American Society for Microbiology, Washington, D.C.
101. **Gholamhoseinian, A., and P. J. Piggot.** 1989. Timing of *spoII* gene expression relative to septum formation during sporulation of *Bacillus subtilis*. J. Bacteriol. **171:**5747–5749.
102. **Gholamhoseinian, A., Z. Shen, J.-J. Wu, and P. Piggot.** 1992. Regulation of transcription of the cell division gene *ftsA* during sporulation of *Bacillus subtilis*. J. Bacteriol. **174:**4647–4656.
103. **Gonzy-Tréboul, G., C. Karmazyn-Campelli, and P. Stragier.** 1992. Developmental regulation of transcription of the *Bacillus subtilis ftsAZ* operon. J. Mol. Biol. **224:**967–979.

104. **Gould, G. W.** 1984. Mechanisms of resistance and dormancy, p. 173–209. *In* A. Hurst and G. W. Gould (ed.), The bacterial spore, vol. 2. Academic Press Ltd., London.
105. **Grossman, A. D.** 1991. Integration of developmental signals and the initiation of sporulation in B. subtilis. Cell **65**:5–8.
106. **Grossman, A. D., and R. Losick.** 1988. Extracellular control of spore formation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **85**:4369–4373.
107. **Guespin-Michel, J. F.** 1971. Phenotypic reversion in some early blocked sporulation mutants of *Bacillus subtilis*: isolation and phenotype identification of partial revertants. J. Bacteriol. **109**:241–247.
108. **Guespin-Michel, J. F.** 1971. Phenotypic reversion in some early blocked sporulation mutants of *Bacillus subtilis*. Genetic studies of polymyxin-resistant partial revertants. Mol. Gen. Genet. **112**:243–254.
109. **Guzman, P., J. Westpheling, and P. Youngman.** 1988. Characterization of the promoter region of the *Bacillus subtilis spoIIE* operon. J. Bacteriol. **170**:1598–1609.
110. **Hackett, R. H., and P. Setlow.** 1983. Enzymatic activity of precursors of *Bacillus megaterium* spore protease. J. Bacteriol. **153**:375–378.
111. **Haldenwang, W. G., N. Lang, and R. Losick.** 1981. A sporulation-induced sigma-like regulatory protein from B. subtilis. Cell **23**:615–624.
112. **Hanson, R. S., M. V. Curry, and J. V. Garner.** 1972. Mutants of *Bacillus cereus* strain T that produce thermoresistant spores lacking dipicolinate and have low levels of calcium. Can. J. Microbiol. **18**:1139–1143.
113. **Harry, E. J., and R. G. Wake.** 1989. Cloning and expression of a *Bacillus subtilis* division initiation gene for which a homolog has not been identified in another organism. J. Bacteriol. **171**:6835–6839.
114. **Hartwell, L. H., and T. A. Weinert.** 1989. Checkpoints: controls that ensure the order of cell cycle events. Science **246**:629–634.
115. **Healy, J., J. Weir, I. Smith, and R. Losick.** 1991. Post-transcriptional control of a sporulation regulatory gene encoding transcription factor σ^H in *Bacillus subtilis*. Mol. Microbiol. **5**:477–487.
116. **Helmann, J. D., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57**:839–872.
117. **Henriques, A. O.** 1992. Unpublished data.
118. **Henriques, A. O., H. De Lencastre, and P. J. Piggot.** 1992. A *Bacillus subtilis* morphogene cluster that includes *spoVE* is homologous to the *mra* region of *Escherichia coli*. Biochimie **74**:735–748.
119. **Hill, S. H.** 1983. *spoVH* and *spoVJ*—new sporulation loci in *Bacillus subtilis* 168. J. Gen. Microbiol. **129**:293–302.
120. **Hitchins, A. D., and R. A. Slepecky.** 1969. Bacterial spore formation as a modified procaryotic cell division. Nature (London) **223**:804–807.
121. **Hoch, J. A., K. Trach, F. Kawamura, and H. Saito.** 1985. Identification of the transcriptional suppressor *sof-1* as an alteration in the *spo0A* protein. J. Bacteriol. **161**:552–555.
122. **Hogarth, C., and D. J. Ellar.** 1979. Energy-dependence of calcium accumulation during sporulation of *Bacillus megaterium* KM. Biochem. J. **178**:627–632.
123. **Holland, S. K., S. Cutting, and J. Mandelstam.** 1987. The possible DNA-binding nature of the regulatory proteins, encoded by *spoIID* and *gerE*, involved in the sporulation of *Bacillus subtilis*. J. Gen. Microbiol. **133**:2381–2391.
124. **Hranueli, D., P. J. Piggot, and J. Mandelstam.** 1974. Statistical estimate of the total number of operons specific for *Bacillus subtilis* sporulation. J. Bacteriol. **119**:684–690.
125. **Hulett, F. M., C. Bookstein, C. Edwards, K. Jensen, N. Kapp, and J. Lee.** 1990. Structural similarities and regulation of *Bacillus subtilis* alkaline phosphatases, p. 163–169. *In* M. M. Zukowski, A. T. Ganesan, and J. A. Hoch (ed.), Genetics and biotechnology of bacilli, vol. 3. Academic Press, Inc., San Diego, Calif.
126. **Ikeda, M., T. Sato, M. Wachi, H. K. Jung, F. Ishino, Y. Kobayashi, and M. Matsuhashi.** 1989. Structural similarity among *Escherichia coli* FtsW and RodA proteins and *Bacillus subtilis* SpoVE protein, which function in cell division, cell elongation, and spore formation, respectively. J. Bacteriol. **171**:6375–6378.
127. **Ikeuchi, T., J. Kudoh, and S. Tsunasawa.** 1986. Amino-terminal structure of *spo0A* protein and sequence homology with *spo0F* and *spo0B* proteins. Mol. Gen. Genet. **203**:371–376.
128. **Illing, N., and J. Errington.** 1990. The *spoIIIA* locus is not a major determinant of prespore-specific gene expression during sporulation in *Bacillus subtilis*. J. Bacteriol. **172**:6930–6936.
129. **Illing, N., and J. Errington.** 1991. Genetic regulation of morphogenesis in *Bacillus subtilis*: roles of σ^E and σ^F in prespore engulfment. J. Bacteriol. **173**:3159–3169.
130. **Illing, N., and J. Errington.** 1991. The *spoIIIA* operon of *Bacillus subtilis* defines a new temporal class of mother-cell-specific sporulation genes under the control of the σ^E form of RNA polymerase. Mol. Microbiol. **5**:1927–1940.
131. **Illing, N., and J. Errington.** 1992. Unpublished data.
132. **Illing, N., M. Young, and J. Errington.** 1990. Use of integrational plasmid excision to identify cellular localization of gene expression during sporulation in *Bacillus subtilis*. J. Bacteriol. **172**:6937–6941.
133. **Inoue, T., and T. R. Cech.** 1985. Secondary structure of the circular form of the *Tetrahymena* rRNA intervening sequence: a technique for RNA structure analysis using chemical probes and reverse transcriptase. Proc. Natl. Acad. Sci. USA **82**:648–652.
134. **Ireton, K., and A. D. Grossman.** 1992. Interactions among mutations that cause altered timing of gene expression during sporulation in *Bacillus subtilis*. J. Bacteriol. **174**:3185–3195.
135. **Ishino, F., and M. Matsuhashi.** 1981. Peptidoglycan synthetic enzyme activities of highly purified penicillin-binding protein 3 in *Escherichia coli*: a septum-forming reaction sequence. Biochem. Biophys. Res. Commun. **101**:905–911.
136. **Ito, J.** 1973. Pleiotropic nature of bacteriophage tolerant mutants obtained in early-blocked asporogenous mutants of *Bacillus subtilis* 168. Mol. Gen. Genet. **124**:97–106.
137. **Ito, J., G. Mildner, and J. Spizizen.** 1971. Early-blocked asporogenous mutants of *Bacillus subtilis* 168. I. Isolation and characterization of mutants resistant to antibiotic(s) produced by sporulating *Bacillus subtilis*. Mol. Gen. Genet. **112**:104–109.
138. **Jaacks, K. J., J. Healy, R. Losick, and A. D. Grossman.** 1989. Identification and characterization of genes controlled by the sporulation-regulatory gene *spo0H* in *Bacillus subtilis*. J. Bacteriol. **171**:4121–4129.
139. **James, W., and J. Mandelstam.** 1985. Protease production during sporulation of germination mutants of Bacillus subtilis and the cloning of a functional gerE gene. J. Gen. Microbiol. **131**:2421–2430.
140. **Jenkinson, H. F.** 1981. Germination and resistance defects in spores of a *Bacillus subtilis* mutant lacking a coat polypeptide. J. Gen. Microbiol. **127**:81–91.
141. **Jenkinson, H. F.** 1983. Altered arrangement of proteins in the spore coat of a germination mutant of *Bacillus subtilis*. J. Gen. Microbiol. **129**:1945–1958.
142. **Jenkinson, H. F., D. Kay, and J. Mandelstam.** 1980. Temporal dissociation of late events in *Bacillus subtilis* sporulation from expression of genes that determine them. J. Bacteriol. **141**:793–805.
143. **Jenkinson, H. F., and H. Lord.** 1983. Protease deficiency and its association with defects in spore coat structure, germination and resistance properties in a mutant of *Bacillus subtilis*. J. Gen. Microbiol. **129**:2727–2737.
144. **Jenkinson, H. F., W. D. Sawyer, and J. Mandelstam.** 1981. Synthesis and order of assembly of spore coat proteins in *Bacillus subtilis*. J. Gen. Microbiol. **123**:1–16.
145. **Jonas, R. M., and W. G. Haldenwang.** 1989. Influence of *spo* mutations on σ^E synthesis in *Bacillus subtilis*. J. Bacteriol. **171**:5226–5228.
146. **Jonas, R. M., S. C. Holt, and W. G. Haldenwang.** 1990. Effects of antibiotics on synthesis and persistence of σ^E in sporulating *Bacillus subtilis*. J. Bacteriol. **172**:4616–4623.

147. **Jonas, R. M., H. K. Peters III, and W. G. Haldenwang.** 1990. Phenotypes of *Bacillus subtilis* mutants altered in the precursor-specific region of σ^E. J. Bacteriol. **172:**4178–4186.
148. **Jonas, R. M., E. A. Weaver, T. J. Kenney, C. P. Moran, Jr., and W. G. Haldenwang.** 1988. The *Bacillus subtilis spoIIG* operon encodes both σ^E and a gene necessary for σ^E activation. J. Bacteriol. **170:**507–511.
149. **Joris, B., G. Dive, A. Henriques, P. J. Piggot, and J. M. Ghuysen.** 1990. The life-cycle proteins RodA of *Escherichia coli* and SpoVE of *Bacillus subtilis* have very similar primary structures. Mol. Microbiol. **4:**513–517.
150. **Kapp, N. V., C. W. Edwards, R. S. Chesnut, and F. M. Hulett.** 1990. The *Bacillus subtilis phoAIV* gene: effects of in vitro inactivation on total alkaline phosphatase production. Gene **96:**95–100.
151. **Karmazyn-Campelli, C., C. Bonamy, B. Savelli, and P. Stragier.** 1989. Tandem genes encoding σ-factors for consecutive steps of development in *Bacillus subtilis*. Genes Dev. **3:**150–157.
152. **Kemp, E. H., and A. Moir.** 1992. Unpublished data.
153. **Kemp, E. H., R. L. Sammons, A. Moir, D. Sun, and P. Setlow.** 1991. Analysis of transcriptional control of the *gerD* spore germination gene of *Bacillus subtilis* 168. J. Bacteriol. **173:** 4646–4652.
154. **Kenney, T. J., P. A. Kirchman, and C. P. Moran, Jr.** 1988. Gene encoding σ^E is transcribed from a σ^A-like promoter in *Bacillus subtilis*. J. Bacteriol. **170:**3058–3064.
155. **Kenney, T. J., and C. P. Moran, Jr.** 1987. Organization and regulation of an operon that encodes a sporulation-essential sigma factor in *Bacillus subtilis*. J. Bacteriol. **169:**3329–3339.
156. **Kenney, T. J., K. York, P. Youngman, and C. P. Moran, Jr.** 1989. Genetic evidence that RNA polymerase associated with σ^A factor uses a sporulation-specific promoter in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **86:**9109–9113.
157. **Keynan, A., A. A. Berns, G. Dunn, M. Young, and J. Mandelstam.** 1976. Resporulation of outgrowing *Bacillus subtilis* spores. J. Bacteriol. **128:**8–14.
158. **Keynan, A., and N. Sandler.** 1984. Spore research in historical perspective, p. 1–48. *In* A. Hurst and G. W. Gould (ed.), The bacterial spore, vol. 2. Academic Press Ltd., London.
159. **Kinney, D. M., and M. G. Bramucci.** 1981. Analysis of *Bacillus subtilis* sporulation with spore-converting bacteriophage PMB12. J. Bacteriol. **145:**1281–1285.
160. **Klier, A. F., and G. Rapoport.** 1988. Genetics and regulation of carbohydrate catabolism in *Bacillus*. Annu. Rev. Microbiol. **42:**65–95.
161. **Kobayashi, Y., and H. Anaguchi.** 1985. Cloning, amplification, and characterization of sporulation genes, especially *spoIIG*, of *Bacillus subtilis*, p. 85–94. *In* J. A. Hoch and P. Setlow (ed.), Molecular biology of microbial differentiation. American Society for Microbiology, Washington, D.C.
162. **Kroos, L., B. Kunkel, and R. Losick.** 1989. Switch protein alters specificity of RNA polymerase containing a compartment-specific sigma factor. Science **243:**526–529.
163. **Kunkel, B.** 1991. Compartmentalized gene expression during sporulation in *Bacillus subtilis*. Trends Genet. **7:**167–172.
164. **Kunkel, B., L. Kroos, H. Poth, P. Youngman, and R. Losick.** 1989. Temporal and spatial control of the mother-cell regulatory gene *spoIIID* of *Bacillus subtilis*. Genes Dev. **3:**1735–1744.
165. **Kunkel, B., R. Losick, and P. Stragier.** 1990. The *Bacillus subtilis* gene for the developmental transcription factor σ^K is generated by excision of a dispensable DNA element containing a sporulation recombinase gene. Genes Dev. **4:**525–535.
166. **Kunkel, B., K. Sandman, S. Panzer, P. Youngman, and R. Losick.** 1988. The promoter for a sporulation gene in the *spoIVC* locus of *Bacillus subtilis* and its use in studies of temporal and spatial control of gene expression. J. Bacteriol. **170:**3513–3522.
167. **Kuroda, A., and J. Sekiguchi.** 1990. Cloning, sequencing and genetic mapping of a *Bacillus subtilis* cell wall hydrolase gene. J. Gen. Microbiol. **136:**2209–2216.
168. **LaBell, T. L., J. E. Trempy, and W. G. Haldenwang.** 1987. Sporulation-specific σ factor σ^{29} of *Bacillus subtilis* is synthesized from a precursor protein, P^{31}. Proc. Natl. Acad. Sci. USA **84:**1784–1788.
169. **Lamont, I. L., and J. Mandelstam.** 1984. Identification of a new sporulation locus, *spoIIIF*, in *Bacillus subtilis*. J. Gen. Microbiol. **130:**1253–1261.
170. **Lampel, K. A., B. Uratani, G. R. Chaudhry, R. F. Ramaley, and S. Rudikoff.** 1986. Characterization of the developmentally regulated *Bacillus subtilis* glucose dehydrogenase gene. J. Bacteriol. **166:**238–243.
171. **Lazarevic, V., P. Margot, B. Soldo, and D. Karamata.** 1992. Sequencing and analysis of the *Bacillus subtilis lytRABC* divergon: a regulatory unit encompassing the structural genes of the *N*-acetylmuramoyl-L-alanine amidase and its modifier. J. Gen. Microbiol. **138:**1949–1961.
172. **Lewandoski, M., E. Dubnau, and I. Smith.** 1986. Transcriptional regulation of the *spo0F* gene of *Bacillus subtilis*. J. Bacteriol. **168:**870–877.
173. **Loshon, C. A., and P. Setlow.** 1982. *Bacillus megaterium* spore protease: purification, radioimmunoassay, and analysis of antigen level and localization during growth, sporulation, and spore germination. J. Bacteriol. **150:**303–311.
174. **Loshon, C. A., B. M. Swerdlow, and P. Setlow.** 1982. *Bacillus megaterium* spore protease. Synthesis and processing of precursor forms during sporulation and germination. J. Biol. Chem. **257:**10838–10845.
175. **Losick, R.** 1992. Personal communication.
176. **Losick, R., and J. Pero.** 1981. Cascades of sigma factors. Cell **25:**582–584.
177. **Losick, R., and A. L. Sonenshein.** 1969. Change in the template specificity of RNA polymerase during sporulation in *Bacillus subtilis*. Nature (London) **224:**35–37.
178. **Losick, R., and P. Stragier.** 1992. Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. Nature (London) **355:**601–604.
179. **Losick, R., P. Youngman, and P. J. Piggot.** 1986. Genetics of endospore formation in *Bacillus subtilis*. Annu. Rev. Genet. **20:**625–669.
180. **Lu, S., R. Halberg, and L. Kroos.** 1990. Processing of the mother-cell σ factor, σ^K, may depend on events occurring in the forespore during *Bacillus subtilis* development. Proc. Natl. Acad. Sci. USA **87:**9722–9726.
181. **Mandelstam, J., and S. A. Higgs.** 1974. Induction of sporulation during synchronized chromosome replication in *Bacillus subtilis*. J. Bacteriol. **120:**38–42.
182. **Mandic-Mulec, I., N. Gaur, U. Bai, and I. Smith.** 1992. Sin, a stage-specific repressor of cellular differentiation. J. Bacteriol. **174:**3561–3569.
183. **Margolis, P., A. Driks, and R. Losick.** 1991. Establishment of cell type by compartmentalized activation of a transcription factor. Science **254:**562–565.
184. **Margolis, P. S., A. Driks, and R. Losick.** 1993. Sporulation gene *spoIIB* from *Bacillus subtilis*. J. Bacteriol. **175:**528–540.
185. **Margolis, P., and R. Losick.** 1992. Unpublished data.
186. **Mason, J. M., P. Fajardo-Cavazos, and P. Setlow.** 1988. Levels of mRNAs which code for small, acid-soluble spore proteins and their *lacZ* gene fusions in sporulating cells of *Bacillus subtilis*. Nucleic Acids Res. **16:**6567–6583.
187. **Mason, J. M., R. H. Hackett, and P. Setlow.** 1988. Regulation of expression of genes coding for small, acid-soluble proteins of *Bacillus subtilis* spores: studies using *lacZ* gene fusions. J. Bacteriol. **170:**239–244.
188. **Mason, J. M., and P. Setlow.** 1984. Expression of *Bacillus megaterium* and *Bacillus subtilis* small acid-soluble spore protein genes during stationary-phase growth of asporogenous *B. subtilis* mutants. J. Bacteriol. **157:**931–933.
189. **Mason, J. M., and P. Setlow.** 1986. Essential role of small, acid-soluble spore proteins in resistance of *Bacillus subtilis* spores to UV light. J. Bacteriol. **167:**174–178.
190. **Mason, J. M., and P. Setlow.** 1987. Different small, acid-soluble proteins of the α/β type have interchangeable roles in the heat and UV radiation resistance of *Bacillus subtilis* spores. J. Bacteriol. **169:**3633–3637.

191. **Masuda, E. S., H. Anaguchi, T. Sato, M. Takeuchi, and Y. Kobayashi.** 1990. Nucleotide sequence of the sporulation gene *spoIIGA* from *Bacillus subtilis*. Nucleic Acids Res. **18**:657.
192. **Masuda, E. S., H. Anaguchi, K. Yamada, and Y. Kobayashi.** 1988. Two developmental genes encoding σ factor homologs are arranged in tandem in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **85**:7637–7641.
193. **Mathiopoulos, C., and A. L. Sonenshein.** 1989. Identification of *Bacillus subtilis* genes expressed early during sporulation. Mol. Microbiol. **3**:1071–1081.
194. **Matsuhashi, M., M. Wachi, and F. Ishino.** 1990. Machinery for cell growth and division: penicillin-binding proteins and other proteins. Res. Microbiol. **141**:89–103.
195. **Mitani, T., J. E. Heinze, and E. Freese.** 1977. Induction of sporulation in *Bacillus subtilis* by decoyinine or hadacidin. Biochem. Biophys. Res. Commun. **77**:1118–1125.
196. **Mohr, S. C., N. V. H. A. Sokolov, C. He, and P. Setlow.** 1991. Binding of small acid-soluble spore proteins from *Bacillus subtilis* changes the conformation of DNA from B to A. Proc. Natl. Acad. Sci. USA **88**:77–81.
197. **Moir, A.** 1981. Germination properties of a spore coat-defective mutant of *Bacillus subtilis*. J. Bacteriol. **146**:1106–1116.
198. **Moir, A., and D. A. Smith.** 1990. The genetics of bacterial spore germination. Annu. Rev. Microbiol. **44**:531–553.
199. **Moldover, B., P. J. Piggot, and M. D. Yudkin.** 1991. Identification of the promoter and the transcriptional start site of the *spoVA* operon of *Bacillus subtilis* and *Bacillus licheniformis*. J. Gen. Microbiol. **137**:527–531.
200. **Moran, C. P., Jr.** 1989. Sigma factors and the regulation of transcription, p. 167–184. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of prokaryotic development: a structural and functional analysis of sporulation and germination. American Society for Microbiology, Washington, D.C.
201. **Moran, C. P., Jr., N. Lang, C. D. B. Banner, W. G. Haldenwang, and R. Losick.** 1981. Promoter for a developmentally regulated gene in *Bacillus subtilis*. Cell **25**:783–791.
202. **Murrell, W. G., and A. D. Warth.** 1965. Composition and heat resistance of bacterial spores, p. 1–24. *In* L. L. Campbell and H. O. Halvorson (ed.), Spores III. American Society for Microbiology, Washington, D.C.
203. **Mysliwiec, T. H., J. Errington, A. B. Vaidya, and M. G. Bramucci.** 1991. The *Bacillus subtilis spo0J* gene: evidence for involvement in catabolite repression of sporulation. J. Bacteriol. **173**:1911–1919.
204. **Nakatani, Y., W. L. Nicholson, K.-D. Neitzke, P. Setlow, and E. Freese.** 1989. Sigma-G RNA polymerase controls forespore-specific expression of the glucose dehydrogenase operon in *Bacillus subtilis*. Nucleic Acids Res. **17**:999–1017.
205. **Nicholson, W. L., B. Setlow, and P. Setlow.** 1990. Binding of DNA in vitro by a small, acid-soluble spore protein from *Bacillus subtilis* and the effect of this binding on DNA topology. J. Bacteriol. **172**:6900–6906.
206. **Nicholson, W. L., and P. Setlow.** 1990. Dramatic increase in negative superhelicity of plasmid DNA in the forespore compartment of sporulating cells of *Bacillus subtilis*. J. Bacteriol. **172**:7–14.
207. **Nicholson, W. L., D. Sun, B. Setlow, and P. Setlow.** 1989. Promoter specificity of σ^G-containing RNA polymerase from sporulating cells of *Bacillus subtilis*: identification of a group of forespore-specific promoters. J. Bacteriol. **171**:2708–2718.
208. **Panzer, S., R. Losick, D. Sun, and P. Setlow.** 1989. Evidence for an additional temporal class of gene expression in the forespore compartment of sporulating *Bacillus subtilis*. J. Bacteriol. **171**:561–564.
209. **Partridge, S. R., and J. Errington.** Importance of morphological events and intercellular interactions in the regulation of prespore-specific gene expression during sporulation in *Bacillus subtilis*. Submitted for publication.
210. **Partridge, S. R., and J. Errington.** Unpublished data.
211. **Partridge, S. R., D. Foulger, and J. Errington.** 1991. The role of σ^F in prespore-specific transcription in *Bacillus subtilis*. Mol. Microbiol. **5**:757–767.
212. **Perego, M., S. P. Cole, D. Burbulys, K. Trach, and J. A. Hoch.** 1989. Characterization of the gene for a protein kinase which phosphorylates the sporulation-regulatory proteins Spo0A and Spo0F of *Bacillus subtilis*. J. Bacteriol. **171**:6187–6196.
213. **Perego, M., C. F. Higgins, S. R. Pearce, M. P. Gallagher, and J. A. Hoch.** 1991. The oligopeptide transport system of *Bacillus subtilis* plays a role in the initiation of sporulation. Mol. Microbiol. **5**:173–185.
214. **Perego, M., and J. A. Hoch.** 1987. Isolation and sequence of the *spo0E* gene: its role in initiation of sporulation in *Bacillus subtilis*. Mol. Microbiol. **1**:125–132.
215. **Perego, M., and J. A. Hoch.** 1988. Sequence analysis and regulation of the *hpr* locus, a regulatory gene for protease production and sporulation in *Bacillus subtilis*. J. Bacteriol. **170**:2560–2567.
216. **Perego, M., and J. A. Hoch.** 1991. Negative regulation of *Bacillus subtilis* sporulation by the *spo0E* gene product. J. Bacteriol. **173**:2514–2520.
217. **Perego, M., G. B. Spiegelman, and J. A. Hoch.** 1988. Structure of the gene for the transition state regulator, *abrB*: regulator synthesis is controlled by the *spo0A* sporulation gene in *Bacillus subtilis*. Mol. Microbiol. **2**:689–699.
218. **Perego, M., J.-J. Wu, G. B. Spiegelman, and J. A. Hoch.** 1991. Mutational dissociation of the positive and negative regulatory properties of the Spo0A sporulation transcription factor of *Bacillus subtilis*. Gene **100**:207–212.
219. **Piggot, P. J.** 1973. Mapping of asporogenous mutations of *Bacillus subtilis*: a minimum estimate of the number of sporulation operons. J. Bacteriol. **114**:1241–1253.
220. **Piggot, P. J., M. Amjad, J.-J. Wu, H. Sandoval, and J. Castro.** 1990. Genetic and physical maps of *Bacillus subtilis* 168, p. 493–543. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley & Sons Ltd., Chichester, United Kingdom.
221. **Piggot, P. J., and J. G. Coote.** 1976. Genetic aspects of bacterial endospore formation. Bacteriol. Rev. **40**:908–962.
222. **Piggot, P. J., C. A. M. Curtis, and H. De Lencastre.** 1984. Use of integrational plasmid vectors to demonstrate the polycistronic nature of a transcriptional unit (*spoIIA*) required for sporulation of *Bacillus subtilis*. J. Gen. Microbiol. **130**:2123–2136.
223. **Piggot, P. J., A. Moir, and D. A. Smith.** 1981. Advances in the genetics of *Bacillus subtilis* differentiation, p. 29–39. *In* H. S. Levinson, A. L. Sonenshein, and D. J. Tipper (ed.), Sporulation and germination. American Society for Microbiology, Washington, D.C.
224. **Popham, D. L., and P. Stragier.** 1991. Cloning, characterization, and expression of the *spoVB* gene of *Bacillus subtilis*. J. Bacteriol. **173**:7942–7949.
225. **Porter, A. C. G., and J. Mandelstam.** 1982. A mutant of *Bacillus subtilis* secreting a DNAase inhibitor during sporulation. J. Gen. Microbiol. **128**:1903–1914.
226. **Predich, M., G. Nair, and I. Smith.** 1992. *Bacillus subtilis* early sporulation genes *kinA*, *spo0F*, and *spo0A* are transcribed by the RNA polymerase containing σ^H. J. Bacteriol. **174**:2771–2778.
227. **Rather, P. N., R. Coppolecchia, H. DeGrazia, and C. P. Moran, Jr.** 1990. Negative regulator of σ^G-controlled gene expression in stationary-phase *Bacillus subtilis*. J. Bacteriol. **172**:709–715.
228. **Rather, P. N., and C. P. Moran, Jr.** 1988. Compartment-specific transcription in *Bacillus subtilis*: identification of the promoter for *gdh*. J. Bacteriol. **170**:5086–5092.
229. **Reeve, J. N., N. H. Mendelson, S. I. Coyne, L. L. Hallock, and R. M. Cole.** 1973. Minicells of *Bacillus subtilis*. J. Bacteriol. **114**:860–873.
230. **Rico, E., S. Cutting, and R. Losick.** 1992. Characterization of *bofA*, a gene involved in intercompartmental regulation of pro-σ^K processing during sporulation in *Bacillus subtilis*. J. Bacteriol. **174**:3177–3184.
231. **Robertson, J. B., M. Gocht, M. A. Marahiel, and P. Zuber.** 1989. AbrB, a regulator of gene expression in *Bacillus*, interacts with the transcription initiation regions of a sporulation gene and an antibiotic biosynthesis gene. Proc. Natl. Acad. Sci. USA **86**:8457–8461.

232. **Roels, S., A. Driks, and R. Losick.** 1992. Characterization of *spoIVA*, a sporulation gene involved in coat morphogenesis in *Bacillus subtilis*. J. Bacteriol. **174:**575–585.
233. **Rong, S., M. S. Rosenkrantz, and A. L. Sonenshein.** 1986. Transcriptional control of the *Bacillus subtilis spoIID* gene. J. Bacteriol. **165:**771–779.
234. **Rosenbluh, A., C. D. B. Banner, R. Losick, and P. C. Fitz-James.** 1981. Identification of a new developmental locus in *Bacillus subtilis* by construction of a deletion mutation in a cloned gene under sporulation control. J. Bacteriol. **148:**341–351.
235. **Rudner, D. Z., J. R. LeDeaux, K. Ireton, and A. D. Grossman.** 1991. The *spo0K* locus of *Bacillus subtilis* is homologous to the oligopeptide permease locus and is required for sporulation and competence. J. Bacteriol. **173:**1388–1398.
236. **Ryter, A.** 1965. Etude morphologique de la sporulation de *Bacillus subtilis*. Ann. Inst. Pasteur (Paris) **108:**40–60.
237. **Sammons, R. L., A. Moir, and D. A. Smith.** 1981. Isolation and properties of spore germination mutants of *Bacillus subtilis* 168 deficient in the initiation of germination. J. Gen. Microbiol. **124:**229–241.
238. **Sammons, R. L., G. M. Slynn, and D. A. Smith.** 1987. Genetical and molecular studies on *gerM*, a new developmental locus of *Bacillus subtilis*. J. Gen. Microbiol. **133:**3299–3312.
239. **Sandman, K., L. Kroos, S. Cutting, P. Youngman, and R. Losick.** 1988. Identification of the promoter for a spore coat protein gene in *Bacillus subtilis* and studies on the regulation of its induction at a late stage of sporulation. J. Mol. Biol. **200:**461–473.
240. **Sandman, K., R. Losick, and P. Youngman.** 1987. Genetic analysis of *Bacillus subtilis spo* mutations generated by Tn*917*-mediated insertional mutagenesis. Genetics **117:**603–617.
241. **Sastry, K. J., O. P. Srivastava, J. Millet, P. C. Fitz-James, and A. I. Aronson.** 1983. Characterization of *Bacillus subtilis* mutants with a temperature-sensitive intracellular protease. J. Bacteriol. **153:**511–519.
242. **Sato, T., Y. Samori, and Y. Kobayashi.** 1990. The *cisA* cistron of *Bacillus subtilis* sporulation gene *spoIVC* encodes a protein homologous to a site-specific recombinase. J. Bacteriol. **172:** 1092–1098.
243. **Sato, T., G. Theeragool, T. Yamamoto, M. Okamoto, and Y. Kobayashi.** 1990. Revised nucleotide sequence of the sporulation gene *spoVE* from *Bacillus subtilis*. Nucleic Acids Res. **18:**4021.
244. **Satola, S., P. A. Kirchman, and C. P. Moran, Jr.** 1991. Spo0A binds to a promoter used by σ^A RNA polymerase during sporulation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **88:**4533–4537.
245. **Satola, S. W., J. M. Baldus, and C. P. Moran, Jr.** 1992. Binding of Spo0A stimulates *spoIIG* promoter activity in *Bacillus subtilis*. J. Bacteriol. **174:**1448–1453.
246. **Savva, D., and J. Mandelstam.** 1986. Synthesis of *spoIIA* and *spoVA* mRNA in *Bacillus subtilis*. J. Gen. Microbiol. **132:** 3005–3011.
247. **Schmidt, R., P. Margolis, L. Duncan, R. Coppolecchia, C. P. Moran, Jr., and R. Losick.** 1990. Control of developmental transcription factor σ^F by sporulation regulatory proteins SpoIIAA and SpoIIAB in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **87:**9221–9225.
248. **Sekiguchi, J., B. Ezaki, K. Kodama, and T. Akamatsu.** 1988. Molecular cloning of a gene affecting the autolysin level and flagellation in *Bacillus subtilis*. J. Gen. Microbiol. **134:**1611–1621.
249. **Setlow, B., A. R. Hand, and P. Setlow.** 1991. Synthesis of a *Bacillus subtilis* small, acid-soluble spore protein in *Escherichia coli* causes cell DNA to assume some characteristics of spore DNA. J. Bacteriol. **173:**1642–1653.
250. **Setlow, B., N. Magill, P. Febbroriello, L. Nakhimovsky, D. E. Koppel, and P. Setlow.** 1991. Condensation of the forespore nucleoid early in sporulation of *Bacillus* species. J. Bacteriol. **173:**6270–6278.
251. **Setlow, B., and P. Setlow.** 1987. Thymine-containing dimers as well as spore photoproducts are found in ultraviolet-irradiated *Bacillus subtilis* spores that lack small acid-soluble proteins. Proc. Natl. Acad. Sci. USA **84:**421–423.
252. **Setlow, P.** 1981. Biochemistry of bacterial forespore development and spore germination, p. 13–28. *In* H. S. Levinson, A. L. Sonenshein, and D. J. Tipper (ed.), Sporulation and germination. American Society for Microbiology, Washington, D.C.
253. **Setlow, P.** 1984. Germination and outgrowth, p. 211–254. *In* A. Hurst and G. W. Gould (ed.), The bacterial spore, vol. 2. Academic Press Ltd., London.
254. **Setlow, P.** 1988. Small, acid-soluble spore proteins of *Bacillus* species: structure, synthesis, genetics, function, and degradation. Annu. Rev. Microbiol. **42:**319–338.
255. **Setlow, P.** 1992. I will survive: protecting and repairing spore DNA. J. Bacteriol. **174:**2737–2741.
256. **Siegele, D. A., J. C. Hu, W. A. Walter, and C. A. Gross.** 1989. Altered promoter recognition by mutant forms of the σ^{70} subunit of *Escherichia coli* RNA polymerase. J. Mol. Biol. **206:**591–603.
257. **Silver-Mysliwiec, T. H., and M. G. Bramucci.** 1990. Bacteriophage-enhanced sporulation: comparison of spore-converting bacteriophages PMB12 and SP10. J. Bacteriol. **172:**1948–1953.
258. **Singh, R. P., B. Setlow, and P. Setlow.** 1977. Levels of small molecules and enzymes in the mother cell compartment and the forespore of sporulating *Bacillus megaterium*. J. Bacteriol. **130:**1130–1138.
259. **Slack, F. J., J. P. Mueller, M. A. Strauch, C. Mathiopoulos, and A. L. Sonenshein.** 1991. Transcriptional regulation of a *Bacillus subtilis* dipeptide transport operon. Mol. Microbiol. **5:**1915–1925.
260. **Sloma, A., G. A. Rufo, Jr., C. F. Rudolph, B. J. Sullivan, K. A. Theriault, and J. Pero.** 1990. Bacillopeptidase F of *Bacillus subtilis*: purification of the protein and cloning of the gene. J. Bacteriol. **172:**5520–5521. (Erratum.)
261. **Slynn, G. M., B. Corfe, and D. A. Smith.** 1992. Unpublished data.
262. **Sonenshein, A. L.** 1989. Metabolic regulation of sporulation and other stationary-phase phenomena, p. 109–130. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of prokaryotic development: a structural and functional analysis of sporulation and germination. American Society for Microbiology, Washington, D.C.
263. **Sowell, M. O., and C. E. Buchanan.** 1983. Changes in penicillin-binding proteins during sporulation of *Bacillus subtilis*. J. Bacteriol. **153:**1331–1337.
264. **Spiegelman, G., B. Van Hoy, M. Perego, J. Day, K. Trach, and J. A. Hoch.** 1990. Structural alterations in the *Bacillus subtilis* Spo0A regulatory protein which suppress mutations at several *spo0* loci. J. Bacteriol. **172:**5011–5019.
265. **Stephens, M. A., N. Lang, K. Sandman, and R. Losick.** 1984. A promoter whose utilization is temporally regulated during sporulation in *Bacillus subtilis*. J. Mol. Biol. **176:**333–348.
266. **Sterlini, J. M., and J. Mandelstam.** 1969. Commitment to sporulation in *Bacillus subtilis* and its relationship to the development of actinomycin resistance. Biochem. J. **113:**29–37.
267. **Stevens, C. M., R. Daniel, N. Illing, and J. Errington.** 1992. Characterization of a sporulation gene, *spoIVA*, involved in spore coat morphogenesis in *Bacillus subtilis*. J. Bacteriol. **174:**586–594.
268. **Stevens, C. M., and J. Errington.** 1990. Differential gene expression during sporulation in *Bacillus subtilis*: structure and regulation of the *spoIIID* gene. Mol. Microbiol. **40:**543–551.
269. **Stock, J. B., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53:**450–490.
270. **Stragier, P.** 1986. Comment on "Duplicated sporulation genes in bacteria" by J. Errington, P. Fort, and J. Mandelstam. FEBS Lett. **195:**9–11.
271. **Stragier, P.** 1989. Temporal and spatial control of gene expression during sporulation: from facts to speculations, p. 243–254. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of prokaryotic development: a structural and functional analysis

of sporulation and germination. American Society for Microbiology, Washington, D.C.
272. **Stragier, P.** 1992. Establishment of forespore-specific gene expression during sporulation of *Bacillus subtilis*, p. 297–310. *In* S. Mohan, C. Dow, and J. A. Cole (ed.), Prokaryotic structure and function: a new perspective. Cambridge University Press, Cambridge.
273. **Stragier, P., C. Bonamy, and C. Karmazyn-Campelli.** 1988. Processing of a sporulation sigma factor in *Bacillus subtilis*: how morphological structure could control gene expression. Cell **52**:697–704.
274. **Stragier, P., J. Bouvier, C. Bonamy, and J. Szulmajster.** 1984. A developmental gene product of *Bacillus subtilis* homologous to the sigma factor of *Escherichia coli*. Nature (London) **312**:376–378.
275. **Stragier, P., B. Kunkel, L. Kroos, and R. Losick.** 1989. Chromosomal rearrangement generating a composite gene for a developmental transcription factor. Science **243**:507–512.
276. **Stragier, P., and R. Losick.** 1990. Cascades of sigma factors revisited. Mol. Microbiol. **4**:1801–1806.
277. **Strauch, M., V. Webb, G. Spiegelman, and J. A. Hoch.** 1990. The Spo0A protein of *Bacillus subtilis* is a repressor of the *abrB* gene. Proc. Natl. Acad. Sci. USA **87**:1801–1805.
278. **Strauch, M. A., M. Perego, D. Burbulys, and J. A. Hoch.** 1989. The transition state transcription regulator AbrB of *Bacillus subtilis* is autoregulated during vegetative growth. Mol. Microbiol. **3**:1203–1209.
279. **Strauch, M. A., G. B. Spiegelman, M. Perego, W. C. Johnson, D. Burbulys, and J. A. Hoch.** 1989. The transition state transcription regulator *abrB* of *Bacillus subtilis* is a DNA binding protein. EMBO J. **8**:1615–1621.
280. **Sun, D., R. M. Cabrera-Martinez, and P. Setlow.** 1991. Control of transcription of the *Bacillus subtilis spoIIIG* gene, which codes for the forespore-specific transcription factor σ^G. J. Bacteriol. **173**:2977–2984.
281. **Sun, D., P. Fajardo-Cavazos, M. D. Sussman, F. Tovar-Rojo, R.-M. Cabrera-Martinez, and P. Setlow.** 1991. Effect of chromosome location of *Bacillus subtilis* forespore genes on their *spo* gene dependence and transcription by $E\sigma^F$: identification of features of good $E\sigma^F$-dependent promoters. J. Bacteriol. **173**:7867–7874.
282. **Sun, D., and P. Setlow.** 1991. Cloning, nucleotide sequence, and expression of the *Bacillus subtilis ans* operon, which codes for L-asparaginase and L-aspartase. J. Bacteriol. **173**:3831–3845.
283. **Sun, D., P. Stragier, and P. Setlow.** 1989. Identification of a new σ-factor involved in compartmentalized gene expression during sporulation of *Bacillus subtilis*. Genes Dev. **3**:141–149.
284. **Sussman, M. D., and P. Setlow.** 1991. Cloning, nucleotide sequence, and regulation of the *Bacillus subtilis gpr* gene, which codes for the protease that initiates degradation of small, acid-soluble proteins during sporulation. J. Bacteriol. **173**:291–300.
285. **Tapsco, H., R. L. Davis, M. J. Thayer, P.-F. Cheng, H. Weintraub, and A. B. Lassar.** 1988. MyoD1: a nuclear phosphoprotein requiring a Myc homology region to convert fibroblasts to myoblasts. Science **242**:405–411.
286. **Tatti, K. M., C. H. Jones, and C. P. Moran, Jr.** 1991. Genetic evidence for interaction of σ^E with the *spoIIID* promoter in *Bacillus subtilis*. J. Bacteriol. **173**:7828–7833.
287. **Tipper, D. J., and J. J. Gauthier.** 1972. Structure of the bacterial endospore, p. 3–12. *In* H. O. Halvorson, R. Hanson, and L. L. Campbell (ed.), Spores V. American Society for Microbiology, Washington, D.C.
288. **Tipper, D. J., and P. E. Linnett.** 1976. Distribution of peptidoglycan synthetase activities between sporangia and forespores in sporulating cells of *Bacillus sphaericus*. J. Bacteriol. **126**:213–221.
289. **Todd, J. A., E. J. Bone, and D. J. Ellar.** 1985. The sporulation-specific penicillin-binding protein 5a from *Bacillus subtilis* is a DD-carboxypeptidase *in vitro*. Biochem. J. **230**:825–828.
290. **Todd, J. A., E. J. Bone, P. J. Piggot, and D. J. Ellar.** 1983. Differential expression of penicillin-binding protein structural genes during *Bacillus subtilis* sporulation. FEMS Microbiol. Lett. **18**:197–202.
291. **Todd, J. A., A. N. Roberts, K. Johnstone, P. J. Piggot, G. Winter, and D. J. Ellar.** 1986. Reduced heat resistance of mutant spores after cloning and mutagenesis of the *Bacillus subtilis* gene encoding penicillin-binding protein 5. J. Bacteriol. **167**:257–264.
292. **Trach, K., J. W. Chapman, P. Piggot, D. LeCoq, and J. A. Hoch.** 1988. Complete sequence and transcriptional analysis of the *spo0F* region of the *Bacillus subtilis* chromosome. J. Bacteriol. **170**:4194–4208.
293. **Trach, K., and J. A. Hoch.** 1989. The *Bacillus subtilis spo0B* stage 0 sporulation operon encodes an essential GTP-binding protein. J. Bacteriol. **171**:1362–1371.
294. **Trempy, J. E., C. Bonamy, J. Szulmajster, and W. G. Haldenwang.** 1985. *Bacillus subtilis* σ factor σ^{29} is the product of the sporulation-essential gene *spoIIG*. Proc. Natl. Acad. Sci. USA **82**:4189–4192.
295. **Trempy, J. E., J. Morrison-Plummer, and W. G. Haldenwang.** 1985. Synthesis of σ^{29}, an RNA polymerase specificity determinant, is a developmentally regulated event in *Bacillus subtilis*. J. Bacteriol. **161**:340–346.
296. **Trowsdale, J., S. M. H. Chen, and J. A. Hoch.** 1979. Genetic analysis of a class of polymyxin resistant partial revertants of stage 0 sporulation mutants of *Bacillus subtilis*: map of the chromosome region near the origin of replication. Mol. Gen. Genet. **173**:61–70.
297. **Turner, S. M., J. Errington, and J. Mandelstam.** 1986. Use of a *lacZ* gene fusion to determine the dependence pattern of sporulation operon *spoIIIC* in *spo* mutants of *Bacillus subtilis*: a branched pathway of expression of sporulation operons. J. Gen. Microbiol. **132**:2995–3003.
298. **Valle, F., and E. Ferrari.** 1989. Subtilisin: a redundantly temporally regulated gene? p. 131–146. *In* I. Smith, R. A. Slepecky, and P. Setlow (ed.), Regulation of prokaryotic development: a structural and functional analysis of sporulation and germination. American Society for Microbiology, Washington, D.C.
299. **Van Hoy, B. E., and J. A. Hoch.** 1990. Characterization of the *spoIVB* and *recN* loci of *Bacillus subtilis*. J. Bacteriol. **172**: 1306–1311.
300. **Van Sinderen, D.** 1992. Unpublished data.
301. **Waites, W. M., D. Kay, I. W. Dawes, D. A. Wood, S. C. Warren, and J. Mandelstam.** 1970. Sporulation in *Bacillus subtilis*. Correlation of biochemical events with morphological changes in asporogenous mutants. Biochem. J. **118**:667–676.
302. **Warburg, R. J., C. E. Buchanan, K. Parent, and H. O. Halvorson.** 1986. A detailed study of *gerJ* mutants of *Bacillus subtilis*. J. Gen. Microbiol. **132**:2309–2319.
303. **Ward, J. E., Jr., and J. Lutkenhaus.** 1985. Overproduction of FtsZ induces minicell formation in *E. coli*. Cell **42**:941–949.
304. **Warren, S. C.** 1968. Sporulation in *Bacillus subtilis*. Biochemical changes. Biochem. J. **109**:811–818.
305. **Warth, A. D., and J. L. Strominger.** 1972. Structure of the peptidoglycan from spores of *Bacillus subtilis*. Biochemistry **11**:1389–1396.
306. **Waxman, D. J., and J. L. Strominger.** 1983. Penicillin-binding proteins and the mechanism of action of beta-lactam antibiotics. Annu. Rev. Biochem. **52**:825–869.
307. **Weir, J., M. Predich, E. Dubnau, G. Nair, and I. Smith.** 1991. Regulation of *spo0H*, a gene coding for the *Bacillus subtilis* σ^H factor. J. Bacteriol. **173**:521–529.
308. **Wu, J.-J., M. G. Howard, and P. J. Piggot.** 1989. Regulation of transcription of the *Bacillus subtilis spoIIA* locus. J. Bacteriol. **171**:692–698.
309. **Wu, J.-J., P. J. Piggot, K. M. Tatti, and C. P. Moran, Jr.** 1991. Transcription of the *Bacillus subtilis spoIIA* locus. Gene **101**: 113–116.
310. **Wu, J.-J., R. Schuch, and P. J. Piggot.** 1992. Characterization of a *Bacillus subtilis* sporulation operon that includes genes for an RNA polymerase σ factor and for a putative DD-carboxypeptidase. J. Bacteriol. **174**:4885–4892.
311. **Yamashita, S., F. Kawamura, H. Yoshikawa, H. Takahashi, Y. Kobayashi, and H. Saito.** 1989. Dissection of the expression

signals of the *spo0A* gene of *Bacillus subtilis*: glucose represses sporulation-specific expression. J. Gen. Microbiol. **135:**1335–1345.

312. **Yamashita, S., H. Yoshikawa, F. Kawamura, H. Takahashi, T. Yamamoto, Y. Kobayashi, and H. Saito.** 1986. The effect of *spo0* mutations on the expression of *spo0A*- and *spo0F-lacZ* fusions. Mol. Gen. Genet. **205:**28–33.
313. **York, K., T. J. Kenney, S. Satola, C. P. Moran, Jr., H. Poth, and P. Youngman.** 1992. Spo0A controls the σ^A-dependent activation of *Bacillus subtilis* sporulation-specific transcription unit *spoIIE*. J. Bacteriol. **174:**2648–2658.
314. **Young, M.** 1976. Use of temperature-sensitive mutants to study gene expression during sporulation in *Bacillus subtilis*. J. Bacteriol. **126:**928–936.
315. **Young, M., and J. Mandelstam.** 1979. Early events during bacterial endospore formation. Adv. Microb. Physiol. **20:**103–162.
316. **Youngman, P.** 1990. Use of transposons and integrational vectors for mutagenesis and construction of gene fusions in *Bacillus* species, p. 221–266. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular biological methods for *Bacillus*. John Wiley & Sons, Ltd., Chichester, United Kingdom.
317. **Youngman, P.** 1992. Unpublished data.
318. **Yudkin, M. D.** 1986. The sigma-like product of sporulation gene *spoIIAC* of *Bacillus subtilis* is toxic to *Escherichia coli*. Mol. Gen. Genet. **202:**55–57.
319. **Yudkin, M. D., and D. Harrison.** 1987. Effect of precisely identified mutations in the *spoIIAC* gene of *Bacillus subtilis* on the toxicity of the sigma-like gene product to *Escherichia coli*. Mol. Gen. Genet. **209:**333–334.
320. **Yura, T., H. Mori, H. Nagai, T. Nagata, A. Ishihama, N. Fujita, K. Isono, K. Mizobuchi, and A. Nakata.** 1992. Systematic sequencing of the *Escherichia coli* genome: analysis of the 0–2.4 min region. Nucleic Acids Res. **20:**3305–3308.
321. **Zheng, L., W. P. Donovan, P. C. Fitz-James, and R. Losick.** 1988. Gene encoding a morphogenic protein required in the assembly of the outer coat of the *Bacillus subtilis* endospore. Genes Dev. **2:**1047–1054.
322. **Zheng, L., R. Halberg, S. Roels, H. Ichikawa, L. Kroos, and R. Losick.** 1992. Sporulation regulatory protein GerE from *Bacillus subtilis* binds to and can activate or repress transcription from promoters for mother-cell-specific genes. J. Mol. Biol. **226:**1037–1050.
323. **Zheng, L., and R. Losick.** 1990. Cascade regulation of spore coat gene expression in *Bacillus subtilis*. J. Mol. Biol. **212:**645–660.
324. **Zuber, P., J. Healy, H. L. Carter III, S. Cutting, C. P. Moran, Jr., and R. Losick.** 1989. Mutation changing the specificity of an RNA polymerase sigma factor. J. Mol. Biol. **206:**605–614.
325. **Zuber, P., and R. Losick.** 1983. Use of a *lacZ* fusion to study the role of the *spo0* genes of Bacillus subtilis in developmental regulation. Cell **35:**275–283.
326. **Zuber, P., and R. Losick.** 1987. Role of AbrB in Spo0A- and Spo0B-dependent utilization of a sporulation promoter in *Bacillus subtilis*. J. Bacteriol. **169:**2223–2230.
327. **Zuber, P., M. Marahiel, and J. Robertson.** 1988. Influence of *abrB* on the transcription of the sporulation-associated genes *spoVG* and *spo0H* in *Bacillus subtilis*, p. 123–127. *In* A. T. Ganesan and J. A. Hoch (ed.), Genetics and biotechnology of bacilli, vol. 2. Academic Press, Inc., San Diego, Calif.
328. **Zuberi, A. R., A. Moir, and I. M. Feavers.** 1987. The nucleotide sequence and gene organization of the *gerA* spore germination operon of *Bacillus subtilis* 168. Gene **51:**1–11.